ANA CRISTINA DE MEDEIROS RIBEIRO

Soroproteção reduzida após a vacinação sem adjuvante contra influenza pandêmica A/H1N1 em pacientes com artrite reumatoide

Tese apresentada à Faculdade de Medicina da Universidade de São Paulo para obtenção do título de Doutor em Ciências

Programa de Ciências Médicas

Área de concentração: Processos Imunes e Infecciosos

Orientadora: Profª. Drª. Eloisa Silva Dutra de Oliveira Bonfá

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Porque agora vemos por espelho em enigma, mas então veremos face a face; agora conheço em parte, mas então conhecerei como também sou conhecido.

I Coríntios 13:12

DEDICATÓRIA

Dedico esta tese a todos os que me ensinaram as mais belas facetas do amor:

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Esta tese está de acordo com as seguintes normas, em vigor no momento desta publicação:

Referencias: adaptado de *International Committee of Medical Journals Editors* (Vancouver).

Universidade de São Paulo. Faculdade de Medicina. Serviço de Biblioteca e Documentação. *Guia de apresentação de dissertações, teses e monografias.*
Elaborado por Anneliese Carneiro da Cunha, Maria Julia de A. L. Freddi, Maria F. Crestana, Marinalva de Souza Aragão, Suely Campos Cardoso, Valéria Vilhena. 3ª ed. São Paulo: Divisão de Biblioteca e Documentações; 2012.

Abreviatura dos títulos dos periódicos de acordo com *List of Journals Indexed in Index Medicus*.

SUMÁRIO

LISTA DE ABREVIATURAS E SIGLAS

AR	-	Artrite reumatoide
CAPPesq	-	Comissão de Ética para Análise de Projetos de Pesquisa
DAS28	-	Disease *activity score* com 28 articulações
DMARD	-	Droga antirreumática modificadora de atividade de doença
DP	-	Desvio padrão
EVA	-	Escala visual analógica
FI-MGT	-	Fator de incremento na média geométrica dos títulos
IC	-	Intervalo de confiança
IH	-	Inibição da hemaglutinação
MGT	-	Média geométrica dos títulos
PCR	-	Proteína C reativa
rRT-PCR	-	Reação positiva em cadeia de polimerase de tempo real
SC	-	Taxa de soroconversão
SP	-	Taxa de soroproteção
TNF	-	*Tumoral necrosis factor*
VHS	-	Velocidade de hemossedimentação

LISTA DE TABELAS

RESUMO

Ribeiro ACM. *Soroproteção reduzida após a vacinação sem adjuvante contra a influenza pandêmica A/H1N1 em pacientes com artrite reumatoide* [tese]. São Paulo: Faculdade de Medicina, Universidade de São Paulo; 2013.

Introdução: A vacinação contra a influenza pandêmica A/H1N1 resultou em soroproteção em mais de 85% dos indivíduos saudáveis. Entretanto, dados em pacientes com artrite reumatoide (AR) são escassos. **Objetivos:** O objetivo deste estudo é avaliar a imunogenicidade e a segurança em curto prazo da vacina contra influenza pandêmica A/H1N1 em pacientes com AR e a influência da atividade da doença e da medicação nesta resposta. **Métodos:** Trezentos e quarenta pacientes adultos com AR em seguimento e tratamento regular e 234 controles saudáveis foram examinados antes e 21 dias após receber uma dose da vacina sem adjuvante contra influenza A/California/7/2009. A atividade da doença (DAS28), o tratamento em uso e os títulos de anticorpos também foram avaliados. As taxas de soroproteção (títulos de anticorpos ≥ 1:40) e soroconversão (percentagem de pacientes com aumento de título de anticorpos maior ou igual a 4, se o título pré-vacinal fosse maior ou igual a 1:10; ou título pós-vacinal de pelo menos 1:40, se o título pré-vacinal era menor que 1:10), as médias geométricas dos títulos (MGT) e o fator de incremento das médias geométricas (FI-MGT) foram calculados. Os eventos adversos foram também registrados. **Resultados:** Os pacientes com AR e os controles tinham taxas pré-vacinais de soroproteção (10,8% vs. 11,5%) e MGT (8,0 vs. 9,3) comparáveis ($p>0,05$). Após a vacinação, foi observada redução significativa na resposta dos pacientes com AR *versus* controles ($p<0,001$) em todos os desfechos sorológicos: taxas de soroproteção (60,0 *vs*. 82,9%) e soroconversão (53,2% *vs.* 76,9%), MGT (57,5 *vs.* 122,9) e FI-MGT (7,2 *vs.* 13,2). A atividade de doença não prejudicou a soroproteção ou a soroconversão e se manteve estável em 97,4% dos pacientes. O metotrexato e o abatacepte foram associados à redução da resposta vacinal. A vacinação foi bem tolerada, com poucos efeitos adversos. **Conclusão:** Os dados confirmaram tanto a segurança em curto prazo como, diferente da maioria dos trabalhos com influenza sazonal, a redução da soroproteção em pacientes com AR, não relacionada à atividade de doença e à maioria das medicações em uso (com

exceção do metotrexato e do abatacepte). A extrapolação da resposta imunológica de uma vacina para outra pode não ser possível e estratégias específicas de imunização (possivelmente em duas doses) podem ser necessárias.

Descritores: Vacinação. Vacinas contra Influenza. Formação de Anticorpos. Artrite Reumatoide. Vírus da Influenza A Subtipo H1N1.

SUMMARY

Ribeiro ACM. *Reduced seroprotection after pandemic A/H1N1 influenza adjuvant-free vaccination in patients with rheumatoid arthritis: implications for clinical practice* [thesis]. São Paulo: Faculdade de Medicina, Universidade de São Paulo; 2013.

Background: Pandemic influenza A/H1N1 vaccination yielded seroprotection in more than 85% of healthy individuals. However, similar data are scarce in rheumatoid arthritis (RA) patients. **Objectives:** The objective of this study is to evaluate the immunogenicity and the short-term safety of anti-pandemic influenza A/H1N1 vaccine in RA patients, and the influence of disease activity and medication to the response. **Methods:** Three hundred and forty adult RA patients in regular follow-up and treatment, and 234 healthy controls were assessed before and 21 days after adjuvant-free influenza A/California/7/2009 vaccine. Disease activity (DAS28), current treatment and anti-pandemic influenza A/H1N1 antibody titres were also evaluated. Seroprotection (antibody titre ≥1:40) and seroconversion (the percentage of patients with a fourfold or greater increase in antibody titre, if prevaccination titre was 1:10 or greater, or a postvaccination titre of 1:40 or greater, if prevaccination titre was less than 1:10) rates, geometric mean titres (GMT) and factor increase in geometric mean titre (FI-GMT) were calculated and adverse events registered. **Results:** RA patients and controls showed similar ($p>0.05$) prevaccination seroprotection (10.8% vs. 11.5%) and GMT (8.0 vs. 9.3). After vaccination a significant reduction ($p<0.001$) was observed in all endpoints in RA patients versus controls: seroprotection (60.0 vs. 82.9%; $p<0.0001$) and seroconversion (53.2% vs. 76.9%) rates, GMT (57.5 vs. 122.9) and FI-GMT (7.2 vs. 13.2). Disease activity did not preclude seroprotection or seroconversion and remained unchanged in 97.4% of patients. Methotrexate and abatacept were associated with reduced responses. Vaccination was well tolerated with minimal adverse events. **Conclusions:** The data confirmed both short-term anti-pandemic A/H1N1 vaccine safety and, different from most studies with seasonal influenza, reduced seroprotection in RA patients, unrelated to disease activity and to most medications (except methotrexate and abatacept). Extrapolation of

immune responses from one vaccine to another may therefore not be possible and specific immunization strategies (possibly booster) may be needed.

Descriptors: Vaccination. Influenza Vaccines. Antibody Formation. Arthritis, Rheumatoid. Influenza A Virus, H1N1 Subtype.

1 INTRODUÇÃO

Os benefícios da vacinação para evitar doenças infecciosas em pacientes com doenças inflamatórias autoimunes como a artrite reumatoide (AR) são reconhecidos[1-3]. Entretanto, ainda há preocupação em relação à segurança e à eficácia vacinais nessas populações[2]. Isso é particularmente importante para as doenças pandêmicas emergentes, como a influenza pandêmica A/H1N1. Depois da pandemia da influenza A/H1N1/California/7/2009[4], vacinas monovalentes inativadas foram disponibilizadas através de programas nacionais de vacinação[5], mas com esquemas de vacinação desenvolvidos em indivíduos saudáveis[6]. Recentemente, nosso grupo relatou a resposta à vacina sem adjuvante contra a influenza pandêmica A/H1N1 em uma grande coorte de indivíduos com doenças reumáticas autoimunes, sendo 343 deles com AR[7]. Nesse estudo, de uma forma geral, a vacina demonstrou boa segurança em curto prazo, mas apresentou imunogenicidade reduzida nos pacientes com AR em relação aos controles. Entretanto, não foi abordada a correlação da baixa imunogenicidade com a atividade da AR ou uso de medicação.

O presente estudo avaliou especificamente a influência da atividade clínica da doença e do tratamento com drogas antirreumáticas modificadoras de atividade de doença (DMARDs) em pacientes com AR que receberam a vacina sem adjuvante contra influenza pandêmica A/H1N1.

2 OBJETIVOS

a) Avaliar a imunogenicidade da vacina sem adjuvante contra a influenza pandêmica A/H1N1 em pacientes com AR.

b) Avaliar a influência de fatores potencialmente relacionados à imunogenicidade vacinal, tais como idade, características da doença (atividade e positividade do fator reumatoide) e tratamento.

c) Avaliar a segurança em curto prazo da vacinação sem adjuvante contra a influenza pandêmica A/H1N1 em pacientes com AR, em termos gerais e seu efeito sobre a atividade da doença.

3 MÉTODOS

3.1 Desenho do estudo

O estudo foi prospectivo, em centro único, conduzido durante a campanha nacional de vacinação contra a influenza pandêmica A/H1N1 no Brasil em 2010.

O estudo foi realizado em dois estágios:

a) Vacinação (de 22 de março de 2010 a 2 de abril de 2010);
b) Período de seguimento de 21 dias para cada paciente e controle, a partir do momento da vacinação, com retorno no 21º dia para reavaliação (de 12 a 23 de abril de 2010).

3.2 População do estudo

O estudo comparou dois grupos: um de pacientes com AR e outro de controles saudáveis. Os pacientes, que preenchiam os critérios de 1987 do Colégio Americano de Reumatologia[8], estavam em seguimento regular no ambulatório de reumatologia do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo e foram convocados através de cartas-convite de participação. Dos 562 pacientes convidados, 391 receberam a vacina e 349 completaram as duas fases, com 340 (87% dos vacinados) sendo incluídos no estudo por terem dados sorológicos, clínicos

e terapêuticos disponíveis. Os pacientes não tiveram a dose de sua medicação modificada durante o estudo.

Indivíduos saudáveis foram recrutados no centro de imunização do hospital e constituíram o grupo controle. Dentre os 326 controles vacinados, 234 (71,8%) completaram o estudo.

Todos os pacientes e controles eram maiores de 18 anos e concordaram em assinar o Termo de Consentimento Livre e Esclarecido. O projeto foi aprovado pela Comissão de Ética para Análise de Projetos de Pesquisa do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (CAPPesq) (número 0114/10) e recebeu apoio da Fundação de Amparo à Pesquisa do Estado de São Paulo (2010/10749-0). Foi também registrado no *clinicaltrials.gov* (NCT01151644).

Os critérios de exclusão foram os seguintes:

a) Infecção prévia pelo vírus da influenza pandêmica A/H1N1, confirmada por reação positiva em cadeia de polimerase de tempo real (rRT-PCR) no lavado combinado naso e orofaríngeo ou, em caso de intubação, no aspirado nasotraqueal.
b) Reação anafilática a componentes vacinais ou a proteínas do ovo.
c) Doença infecciosa aguda (temperatura axilar acima de 38ºC) no momento da vacinação.
d) Síndrome de Guillain-Barré ou outras síndromes desmielinizantes.

e) Imunização com qualquer vacina de vírus vivo em até 4 semanas previamente ao estudo.
f) Imunização com qualquer vacina de vírus inativo em até 2 semanas previamente ao estudo.
g) Imunização contra influenza em até 6 meses previamente ao estudo.
h) Hospitalização no momento da inclusão no estudo.
i) Transfusão com hemoderivados até 6 meses antes do estudo.

3.3 Tamanho da Amostra

O cálculo do tamanho da amostra foi feito com base em taxas de soroproteção contra influenza sazonal em populações saudáveis e de pacientes com AR. Admitindo-se um poder de 80%, com erro α de 0,05 em um teste bicaudado, e esperando-se que a população saudável atingisse 75% de soroproteção, contra 60% nos pacientes com AR, o tamanho da amostra seria de 152 pacientes em cada grupo (pacientes e controles). Entretanto, pela indicação formal de vacinação nas populações que constituíram os grupos de estudos, não havendo conflitos éticos, e pela heterogeneidade do uso de medicações nos pacientes com AR, foi incluído no estudo o maior número possível de pacientes e controles disponíveis e que concordaram em participar.

3.4 Vacina contra influenza A/H1N1

A vacina utilizada foi produzida pela Sanofi Pasteur. Na ocasião era uma nova vacina monovalente sem adjuvante (A/California/7/2009/Butantan Institute/Sanofi Pasteur, São Paulo, Brazil). A dose vacinal de 0,5 mL continha 15 µg do antígeno hemaglutinina equivalente à cepa vírus influenza A/California/7/2009 (NYMC X-179A), fragmentada e inativada, recomendada pela OMS. Esta cepa foi propagada em ovos embrionados de galinha, com as mesmas técnicas padronizadas para a produção da vacina sazonal. A vacina foi disponibilizada em frascos multidose de 5 mL, com timerosal como conservante (45 µg por dose de 0,5 mL).

3.5 Procedimentos do Estudo

Todos os pacientes com AR e controles saudáveis foram vacinados com uma dose única intramuscular da vacina. Os pacientes tiveram a atividade de doença avaliada antes da vacinação e 21 dias após, através da medida do DAS28 (*disease activity score* com 28 articulações)[9] e de seus componentes: contagem do número de articulações com edema e com dor, a avalição global da saúde pelo paciente por escala visual analógica (EVA) horizontal de 100 mm, e a medida da velocidade de hemossedimentação (VHS) pelo método de Westergreen. A avaliação da dor pelo paciente e da doença pelo médico, ambas medidas por EVA horizontal de 100 mm, e a medida da proteína C reativa (PCR) por nefelometria também foram realizadas.

O tratamento medicamentoso da AR, incluindo o uso de prednisona e DMARDs não biológicas e biológicas também foi avaliado no momento da vacina, com orientação de não haver modificação do tratamento.

3.6 Avaliação da segurança vacinal

No dia da vacinação, todos os participantes receberam um diário de 21 dias para preenchimento prospectivo, devendo ser entregue 3 semanas depois, no dia da reavaliação. Esse questionário continha uma lista pré-definida de eventos adversos: reações locais (dor, eritema, edema e prurido no local da aplicação da vacina) e eventos adversos sistêmicos (febre, calafrios, artralgia, mialgia e sintomas gripais: dor de garganta, tosse, rinorreia e congestão nasal), além de campos em branco para preenchimento de outros eventos inesperados. No retorno em 21 dias, os pacientes eram ativamente questionados sobre o aparecimento e registro das reações e todos os sintomas novos, registrados ou não no diário, foram avaliados. A relação causal com a vacinação foi determinada pelos pesquisadores: as reações locais foram consideradas como relacionadas à vacina, enquanto os eventos adversos sistêmicos foram analisados para determinar a sua causalidade. Eventos adversos graves foram definidos como aqueles que resultassem em hospitalização ou em óbito.

Além disso, todos os pacientes com AR responderam a uma questão específica e dicotômica (sim ou não) sobre sua percepção sobre a interferência da vacinação na atividade de doença.

3.7 Avaliação laboratorial da imunização

A imunogenicidade contra a vacina foi medida por teste de inibição da hemaglutinação (IH) no Instituto Adolfo Lutz[7,10]. Os títulos de anticorpos e suas médias geométricas (MGTs) foram medidos nos momentos zero e 21 dias após a imunização em todos os participantes. As concentrações de vírus foram previamente determinadas por titulação do antígeno hemaglutinina e o teste de IH foi realizado após a remoção de inibidores não específicos do soro. Os estoques de vírus foram aliquotados e armazenados a -70 º C até sua utilização.

Os soros foram testados para anticorpos contra a cepa do vírus influenza H1N1 A/California/7/2009 fornecido por Butantan a uma diluição inicial de 1:10 e uma diluição final de 1:2560. Para fins de cálculo, um valor de 1:5 foi atribuído para títulos negativos, e um valor de 1:2560 para títulos superiores a 1:2560. Todas as amostras foram testadas em duplicata.

Os desfechos sorológicos primários foram avaliados após 21 dias da vacinação:

a) Taxa de soroproteção (SP): porcentagem de pacientes com títulos de anticorpos $\geq$ 1:40 após a vacinação.
b) Taxa de soroconversão (SC): porcentagem de pacientes com aumento no valor dos títulos de anticorpos $\geq$ 4 se os títulos pré-vacinais fossem $\geq$ 1:10, ou títulos pós-vacinais $\geq$ 1:40, se os títulos pré-vacinais fossem < 1:10.

Os desfechos sorológicos secundários, também avaliados após 21 dias da vacinação, foram:

a) Média geométrica dos títulos (MGT) de anticorpos.
b) Fator de incremento nas médias geométricas dos títulos (FI-MGT): média geométrica da relação entre os títulos de anticorpos pós e pré-vacinais.

A taxa de soroproteção pré-vacinal (porcentagem de pacientes com títulos de anticorpos $\geq$ 1:40 pré-vacinação) e a média geométrica dos títulos (MGT) pré-vacinal também foram avaliadas (momento zero).

3.8 Análise Estatística

As análises de imunogenicidade e de segurança foram descritivas, e intervalos de confiança de 95% (IC) bicaudais foram calculados. As variáveis categóricas (taxas de soroproteção e soroconversão, uso de medicação e a frequência de efeitos adversos) foram comparadas utilizando o teste exato de Fisher ou o Chi-quadrado, conforme apropriado. As MGTs foram comparadas entre pacientes com AR e controles saudáveis utilizando o teste *t-Student* bicaudal ou o teste de Mann-Whitney sobre os títulos transformados em escala logarítmica. Os parâmetros de atividade da doença antes e após a vacinação foram analisados com o teste *t-Student* ou teste de Wilcoxon *rank-sum.* Foram utilizados modelos de regressão múltipla logística para analisar a interação entre as características demográficas, medicações e sua influência na soroproteção e na soroconversão. Todos os testes foram bicaudados, com nível de significância de $p < 0,05$.

4 RESULTADOS

4.1 Dados demográficos e características da AR

Os pacientes com AR e o grupo controle diferiram estatisticamente quanto à média da idade (DP) [55,8 (11,5) *vs.* 36,6 (12,5) anos; p<0,0001] e à frequência de pacientes do sexo feminino (86,7% *vs.* 66,8%; p<0,0001), ambos maiores no grupo de pacientes com AR. Os dados clínicos são descritos na Tabela 1.

4.2 Resposta à imunização

Os grupos de pacientes com AR e controle tinham soroproteção e médias de títulos de anticorpos (MGTs) pré-vacinais semelhantes (p>0,05; Tabela 2). Entretanto, após a vacinação, os pacientes com AR mostraram taxas de soroproteção (SP) e soroconversão (SC) reduzidas em relação aos controles. Apesar do aumento significante das MGTs pós-vacinais em ambos os grupos, a MGT pós-vacinal e o FI-MGT foram menores nos pacientes com AR em comparação aos controles (Tabela 2).

Devido à diferença significante da média de idade entre os grupos originais, foi feita uma subanálise com 88 pacientes com AR e 184 controles [médias de idade (IC): 40,8 (39,4 a 42,1) *vs*. 40,8 anos (39,5 a 42,1); p>0,05]. Nesse subgrupo de idades comparáveis, as MGTs e as frequências

Tabela 1 - Dados demográficos, parâmetros de atividade da doença e tratamento nos pacientes com AR no momento da vacinação

	Pacientes com AR n=340
Dados demográficos	
Sexo feminino, n (%)	295 (86,7)
Idade atual, anos	55,8 (11,5)
Tempo de doença, anos	16,7 (10,4)
Positividade do FR, n (%)	249 (72,8)
Atividade de doença	
Atividade clínica, n(%)	258 (75,9)
DAS28	3,66 (1,35)
Nº de articulações com dor	3,7 (4,8)
Nº de articulações com edema	4,4 (4,6)
VHS, mm/1ª hora	20,7 (20,2)
PCR, mg/dL	12,1 (18,2)
EVA global do paciente, 0-100 mm	37,9 (26,6)
EVA de dor do paciente, 0-100 mm	38,3 (27)
EVA do médico, 0-100 mm	30,6 (26,9)
Tratamento	
Sem medicação, n(%)	11 (3,2)
Prednisona, n(%)	247(72,6)
Dose de prednisona, mg/d	8,6 (5,2)
Metotrexato, n(%)	215(63,2)
Dose de metotrexato, mg/semana	19,2 (5,6)
Leflunomida (dose 20mg/d), n(%)	146 (42,9)
Cloroquina, n(%)	123 (36,2)
Sulfassalazina, n(%)	30 (8,8)
Azatioprina, n(%)	17 (5)
Ciclosporina, n(%)	7 (2,1)
Agentes anti-TNF, n(%)	47(13,8)
Adalimumabe, n(%)	16 (4,7)
Etanercepte, n(%)	11 (3,2)
Infliximabe, n(%)	20 (5,9)
Abatacepte, n(%)	11 (3,2)
Rituximabe, n(%)	12 (3,5)
Tocilizumabe, n(%)	10 (2,9)

Valores expressos em número (%) ou média (DP)

AR = artrite reumatoide; FR = fator reumatoide; DAS28 = *disease activity score* com 28 articulações; atividade clínica = DAS28 > 2,6; VHS = velocidade de hemossedimentação; PCR = proteína C reativa; EVA = escala visual analógica; anti-TNF = anti-*tumoral necrosis factor* ou fator de necrose tumoral.

de soroproteção pré-vacinais também eram similares (p>0,05). Após a vacinação, assim como na análise anterior, os pacientes com AR tiveram menores taxas de soroproteção (SP) (p=0,04) e soroconversão (SC) (p=0,005) em relação aos controles. A MGT pós-vacinal e o FI-MGT foram menores no grupo de pacientes com AR, mas sem atingir significância estatística (p>0,05) (Tabela 2).

Tabela 2 - Imunogenicidade da vacina contra influenza pandêmica A/H1N1 de controles e pacientes com AR nos grupos originais e nos subgrupos com idades comparáveis

	Pré-vacinação		Pós-vacinação			
	MGT	SP, %	MGT	SP, %	FI-MGT	SC, %
Grupos originais						
Controles (n=234)	9,3 (8,2-10,5)	11,5 (7,4-15,6)	122,9 (103,5-146)	82,9 (77,5-87,5)	13,2 (11,1-15,8)	76,9 (71-82,2)
AR (n=340)	8 (7,3-8,8)	10,8 (7,5-14,1)	57,5 (48-68,9)*	60 (54,5-65,3)*	7,2 (6,1-8,5)*	53,2 (47,8-58,6)*
Subgrupos com idades comparáveis						
Controles (n=184)	9 (7,9-10,2)	9,8 (5,5-14,1)	116,6 (96,2-141,3)	83,2 (77,8-88,6)	13 (10,7-15,8)	76,1 (69,9-82,3)
AR (n=88)	8,2 (6,9-9,8)	8 (2,3-13,6)	83,9 (60,3-116,7)	67 (57,2-76,9)*	10,2 (7,5-13,9)	63,6 (53,5-73,7)*

Os dados estão expressos em % ou valor (intervalo de confiança de 95%)

AR = artrite reumatoide; MGT = média geométrica dos títulos; SP = soroproteção; FI-MGT = fator de incremento na MGT após a vacinação; SC = soroconversão; * p <0,05 – comparado ao grupo controle.

4.3 Influência do tratamento

Os pacientes estavam em seguimento ambulatorial regular e recebiam DMARDs tradicionais e/ou biológicas, geralmente em associação. Apenas 11 pacientes (3,2%) estavam sem medicação e 32 (9,4%) estavam em monoterapia. As MGTs e taxas de soroproteção pré-vacinais e todos os desfechos sorológicos pós-vacinais dos pacientes com AR são mostrados na

Tabela 3, de acordo com a positividade do fator reumatoide e o uso de DMARDs ou não. As Tabelas 4 e 5 mostram análises univariadas do uso das medicações tendo os desfechos primários (soroproteção e soroconversão, respectivamente) como variáveis dependentes.

Os pacientes em uso de metotrexato e aqueles em uso de abatacepte tiveram as piores respostas e esses foram os únicos medicamentos que influenciaram negativamente a resposta vacinal no grupo de pacientes com AR em todos os desfechos analisados (Tabelas 3, 4 e 5). Já o uso do rituximabe alterou negativamente apenas desfechos secundários, com menor MGT pós-vacinal e menor FI-MGT (Tabela 3). O número de DMARDs foi maior em pacientes que não soroconverteram, em relação aos dois desfechos primários (Tabelas 4 e 5).

4.4 Influência das características da doença

A soroproteção (Tabela 4) após a vacinação foi influenciada pelo *status* sorológico pré-vacinal e pela MGT prévia. A idade, o fator reumatoide, o tempo de doença e o gênero no grupo de pacientes com AR não influenciaram a soroproteção ($p>0,05$). Em relação ao grau de atividade de doença durante a vacinação, a soroproteção foi associada a maior número de pacientes em atividade, valores maiores de DAS28, maior número de articulações com dor, mas não com edema, e maiores valores de VHS. Já a PCR e as avaliações feitas por EVA foram semelhantes ($p>0,05$) entre pacientes que atingiram soroproteção e os que não atingiram.

Tabela 3 - Imunogenicidade da vacina contra influenza pandêmica A/H1N1 de pacientes com AR de acordo com a positividade do FR e com o tratamento

	Pré-vacinação		Pós-vacinação			
	MGT	SP, %	MGT	SP, %	FI-MGT	SC, %
Fator reumatoide						
Não (n=91)	7,8 (6,5-9,4)	9,6 (3,3-16)	48,1 (33,2-69,6)	54,2 (43,4-65)	6,2 (4,4-8,7)	48,2 (37,4-59)
Sim (n=249)	7,9 (7,1-8,8)	10 (6,3-13,8)	59,6 (48,2-73,6)	64,5 (55,4-67,5)	7,5 (6,2-9,2)	54,2 (48-60,4)
Prednisona						
Não (n=93)	7,8 (7-8,6)	10,1 (6,4-13,9)	58,4 (47,1-72,5)	60,3 (54,2-66,4)	7,5 (6,2-9,2)	54,7 (48,4-60,9)
Sim (n=247)	8,6 (7,2-10,4)	12,9 (6,1-19,7)	55,1 (39,4-77)	59,1 (49-69,2)	6,4 (4,7-8,7)	49,5 (39,2-59,7)
Metotrexato						
Não (n=125)	8 (6,9-9,2)	12,9 (7-12,8)	90,9 (66,3-124,9)*	71,8 (63,8-79,7)*	11,4 (8,4-15,4)*	65,3 (56,9-73,7)*
Sim (n=215)	8,0 (7,1-9)	9,7 (5,8-13,7)	44,2 (35,7-54,7)*	53,2 (46,6-59,9)*	5,5 (4,6-6,7)*	46,3 (39,6-53)*
Leflunomida						
Não (n=194)	8,8 (7,7-9,9)	12,9 (8,2-17,6)	49,2 (39,1-61,9)	56,7 (49,7-63,7)	5,6 (4,6-6,8)	50,5 (43,5-57,6)
Sim (n=146)	7,1 (6,3-7,9)	8,3 (3,8-12,7)	71,3 (53,5-95,1)	64,8 (57,0-72,6)	10,1 (7,6-13,4)	57,2 (49,1-65,3)
Cloroquina						
Não (n=217)	7,6 (6,8-8,5)	10,1 (6,1-14,2)	57,4 (45,4-72,6)	60,8 (54,3-67,3)	7,5 (6,1-9,3)	55,3 (48,7-61,9)
Sim (n=123)	8,7 (7,5-10,1)	12,2 (6,4-18)	57,7 (43,5-76,5)	58,5 (49,8-67,3)	6,6 (5,1-8,7)	49,6 (40,7-58,5)
Sulfassalazina						
Não (n=310)	7,9 (7,2-8,6)	10,3 (6,9-13,7)	55,6 (45,9-67,3)	58,7 (53,2-64,2)	7,1 (5,9-8,4)	52,6 (47-58,2)
Sim (n=30)	9,5 (6,4-14,3)	13,3 (1-25,7)	81,9 (47,9-139,8)	73,3 (57,2-89,4)	8,6 (5-14,6)	60 (42,2-77,8)
Azatioprina						
Não (n=323)	7,9 (7,2-8,7)	10,2 (6,9-13,5)	56,9 (47,3-68,4)	59,8 (54,4-65,1)	7,2 (6-8,5)	52,9 (47,5-58,4)
Sim (n=17)	8,8 (5,5-14,4)	17,7 (0-36,3)	70,8 (28,2-177,5)	64,7 (41,3-88,1)	8 (3,4-18,8)	58,9 (34,7-82,9)
Ciclosporina						
Não (n=333)	8,1 (7,3-8,8)	10,8 (7,5-14,2)	57,2 (47,6-68,8)	59,5 (54,2-64,7)	7,1 (6-8,4)	52,6 (47,2-57,9)
Sim (n=7)	5,5 (4,5-6,7)	0	72,5 (29,2-180,1)	85,7 (57,7-100)	13,1 (5,5-31,5)	85,7 (57,7-100)
Agentes anti-TNF						
Não (n=293)	8,1 (7,2-8,9)	11,2 (7,4-15,0)	55,5 (45,1-68,3)	58,8 (52,9-64,8)	6,8 (5,8-8,3)	51,0 (45,0-57,0)
Sim (n=47)	7,5 (6,1-9,3)	8,7 (0,5-16,9)	71,9 (45,4-114,1)	67,4 (53,7-81,1)	9,6 (5,4-8,1)	67,4 (53,7-81,1)
Adalimumabe						
Não (n=324)	8 (7,3-8,8)	10,8 (7,4-14,2)	58,2 (48,3-70,1)	60,2 (54,9-65,5)	7,2 (6,1-8,6)	53,1 (47,6-58,5)
Sim (n=16)	7,1 (5-10)	6,3 (0-18,5)	45,6 (20,3-102)	56,3 (31,2-81,4)	6,4 (3,2-13,1)	56,3 (31,2-81,4)

Etanercepte						
Não (n=329)	8 (7,3-8,7)	10,3 (7-13,6)	56,9 (47,2-68,5)	60 (54,6-65,2)	7,1 (6-8,5)	52,9 (47,5-58,3)
Sim (n=11)	8,8 (5,5-14,2)	18,2 (0-42,1)	80 (33,9-188,9)	63,6 (33,8-93,5)	9,1 (3,4-24,3)	63,6 (33,8-93,5)
Infliximabe						
Não (n=320)	8,1 (7,3-8,8)	10,9 (7,5-14,4)	56 (46,4-67,5)	59,1 (53,7-64,5)	6,9 (5,9-8,3)	52,2 (46,7-57,7)
Sim (n=20)	7,1 (5,1-9,7)	5 (0-14,8)	88,8 (41,8-188,4)	75 (55,5-94,5)	12,6 (6,1-25,9)	70 (49,4-90,6)
Abatacepte						
Não (n=329)	8,1 (7,3-8,9)	11 (7,6-14,4)	60,8 (50,6-73,1)*	61,7 (56,4-67)*	7,5 (6,4-8,9)*	55 (49,6-60,4)*
Sim (n=11)	6 (4,6-7,9)	0	10,7 (7,2-15,7)*	9,1 (0-26,9)*	1,8 (1,4-2,3)*	0*
Rituximabe						
Não (n=328)	8 (7,3-8,8)	11 (7,5-14,3)	59,6 (49,7-71,6)*	60,7 (55,4-66)	7,4 (6,3-8,8)*	54 (48,6-59,41)
Sim (n=12)	6,7 (4,9-9,1)	0	21,2 (6,9-64,6)*	41,7 (12,5-70,8)	3,2 (1,2-8,1)*	33,3 (5,5-61,2)
Tocilizumabe						
Não (n=330)	8 (7,3-8,8)	10,9 (7,5-14,3)	57 (47,4-68,6)	59,7 (54,4-65)	7,1 (6-8,5)	52,7 (47,3-58,1)
Sim (n=10)	7,6 (5,6-10,2)	0	74,6 (26,3-211,5)	70 (41,5-98,6)	9,8 (3,9-24,9)	70 (41,5-98,6)

Os dados estão expressos em % ou valor (intervalo de confiança de 95%)

AR = artrite reumatoide; MGT = média geométrica dos títulos; SP = soroproteção; FI-MGT = fator de incremento na MGT após a vacinação; SC = soroconversão; TNF = *Tumor Necrosis Factor;* *p <0,05 = comparação entre pacientes com e sem a característica (fator reumatoide ou uso da referida DMARD).

Em relação à soroconversão (Tabela 5), os pacientes que soroconverteram tinham uma média de idade mais baixa que os que não soroconverteram e MGT pré-vacinal mais alta. O fator reumatoide, o tempo de doença e o gênero no grupo de pacientes com AR não influenciaram a resposta vacinal (p>0,05). Em relação ao grau de atividade de doença durante a vacinação, a soroconversão foi associada a valores maiores de DAS28, maior número de articulações com edema e com dor, e maiores valores de VHS. Já a PCR, a frequência de pacientes em atividade e as avaliações feitas por EVA foram semelhantes (p>0,05) entre pacientes conversores e não conversores.

Tabela 4 - Dados demográficos e sorológicos, parâmetros da doença e tratamento de acordo com a soroproteção em pacientes com AR

	Com soroproteção n=204	Sem soroproteção n=136	p
Dados demográficos e sorológicos			
Sexo feminino, n (%)	175 (85,8)	120 (88,2)	0,51
Idade atual, anos	55,3 (11,5)	56,7 (11,5)	0,21
Tempo de doença, anos	16,2 (10,9)	17,4 (9,8)	0,17
Positividade do FR, n (%)	153 (75)	96 (70,6)	0,37
MGT prévia	10,4 (3,8-28,2)	5,4 (4,3-6,9)	<0,001
Soroproteção prévia, n(%)	36 (17,6)	0 (0)	<0,0001
Atividade de doença			
Atividade clínica, n(%)	164 (80,4)	94 (69,1)	0,017
DAS28	3,84 (1,36)	3,39 (1,29)	0,002
Nº de articulações com dor	4,3 (5,2)	2,9 (4,3)	0,006
Nº de articulações com edema	4,8 (4,8)	3,9 (4,3)	0,066
VHS, mm/1ª hora	22,5 (21,2)	18,2 (18,3)	0,047
PCR, mg/dL	12,2 (19,3)	11,8 (15,7)	0,077
EVA global do paciente, 0-100 mm	39,3 (27,7)	36,2 (24,9)	0,47
EVA de dor do paciente, 0-100 mm	39,6 (26,6)	36,7 (27,6)	0,36
EVA do médico, 0-100 mm	32,1 (23,2)	28,5 (22,3)	0,17
Tratamento			
Sem medicação, n(%)	9 (4,4)	2 (1,5)	0,21
Prednisona, n(%)	149 (73)	98 (72,1)	0,84
Dose de prednisona, mg/d	8,9 (5,8)	7,9 (4,3)	0,07
Metotrexato (MTX), n(%)	115 (56,4)	100 (73,5)	0,0013
Dose de MTX, mg/semana	19,2 (5,6)	19,2 (5,6)	0,90
Leflunomida (20mg/d), n(%)	94 (46,1)	52 (38,2)	0,15
Cloroquina, n(%)	72 (35,3)	51 (37,5)	0,68
Sulfassalazina, n(%)	22 (10,8)	8 (5,9)	0,12
Azatioprina, n(%)	11 (5,4)	6 (4,4)	0,68
Ciclosporina, n(%)	6 (2,9)	1 (0,7)	0,25
Agentes anti-TNF, n(%)	31 (15,2)	16 (11,8)	0,29
Adalimumabe, n(%)	9 (4,4)	7 (5,2)	0,75
Etanercepte, n(%)	8 (3,9)	3 (2,2)	0,54
Infliximabe, n(%)	14 (6,9)	6 (4,4)	0,35
Abatacepte, n(%)	1 (0,5)	10 (7,4)	0,0006
Rituximabe, n(%)	5 (2,5)	7 (5,2)	0,19
Tocilizumabe, n(%)	7 (3,4)	3 (2,2)	0,75
Nº de DMARDs	1,3 (0,8)	1,5 (0,8)	0,03

Valores expressos em número (%) ou média (DP)

AR = artrite reumatoide; FR = fator reumatoide; DAS28 = *disease activity score*; atividade clínica = DAS28 > 2,6; VHS = velocidade de hemossedimentação; PCR = proteína C reativa; EVA = escala visual analógica; MTX = metotrexato; anti-TNF = anti-*tumoral necrosis factor*; DMARDs = drogas antirreumáticas modificadoras de atividade de doença.

Tabela 5 - Dados demográficos e sorológicos, parâmetros da doença e tratamento de acordo com a soroconversão em pacientes com AR

	Com soroconversão n=181	Sem soroconversão n=159	p
Dados demográficos e sorológicos			
Sexo feminino, n (%)	156 (86,2)	139 (87,4)	0,74
Idade atual, anos	54,8 (11,4)	57,1 (11,6)	0,022
Tempo de doença, anos	16,2 (10,7	17,3 ± 10,1	0,23
Positividade do FR, n (%)	135 (74,6)	114 (71,7)	0,55
MGT prévia	8,5 (7,5-9,6)	7,5 (6,5-8,6)	0,03
Soroproteção prévia, n(%)	16 (10,1)	20 (11)	0,26
Atividade de doença			
Atividade clínica, n(%)	145 (80,1)	113 (71,1)	0,052
DAS28	3,84 (1,36)	3,45 (1,31)	0,007
Nº de articulações com dor	4,3 (5,2)	3,1 (4,5)	0,012
Nº de articulações com edema	4,9 (4,7)	3,9 (4,5)	0,024
VHS, mm/1ª hora	22,4 (20,8)	18,9 (19,4)	0,04
PCR, mg/dL	11,8 (15,3)	12,3 (20,5)	0,74
EVA global do paciente, 0-100 mm	38,4 (27,2)	37,6 (26)	0,92
EVA de dor do paciente, 0-100 mm	39,3 (26,3)	37,4 (27,8)	0,47
EVA do médico, 0-100 mm	32,8 (23,4)	28,4 (22)	0,11
Tratamento			
Sem medicação, n(%)	7 (3,9)	4 (2,5)	0,55
Prednisona, n(%)	135 (74,1)	112 (70,4)	0,39
Dose de prednisona, mg/d	9,1 (5,9)	7,7 (4,3)	0,07
Metotrexato (MTX), n(%)	100 (55,2)	115 (72,3)	0,001
Dose de MTX, mg/semana	19,2 (5,6)	19,2 (5,6)	0,90
Leflunomida (20mg/d), n(%)	83 (46,6)	63 (39,6)	0,25
Cloroquina, n(%)	61 (33,7)	62 (39)	0,31
Sulfassalazina, n(%)	18 (9,9)	12 (7,5)	0,44
Azatioprina, n(%)	10 (5,5)	7 (4,4)	0,64
Ciclosporina, n(%)	6 (3,3)	1 (0,6)	0,13
Agentes anti-TNF, n(%)	31 (17,2)	16 (10,1)	0,06
Adalimumabe, n(%)	9 (5)	7 (4,4)	0,80
Etanercepte, n(%)	8 (4,4)	3 (1,9)	0,23
Infliximabe, n(%)	14 (7,7)	6 (3,8)	0,12
Abatacepte, n(%)	0 (0)	11 (6,9)	0
Rituximabe, n(%)	4 (2,2)	8 (5)	0,24
Tocilizumabe, n(%)	7 (3,9)	3 (1,9)	0,35
Nº de DMARDs	1,3 (0,8)	1,5 (0,8)	0,03

Valores expressos em número (%) ou média (DP)

AR = artrite reumatoide; FR = fator reumatoide; DAS28 = *disease activity score*; atividade clínica = DAS28 > 2,6; VHS = velocidade de hemossedimentação; PCR = proteína C reativa; EVA = escala visual analógica; MTX = metotrexato; anti-TNF = anti-*tumoral necrosis factor*; DMARDs = drogas antirreumáticas modificadoras de atividade de doença.

4.5 Análises multivariadas

Após análises multivariadas tendo a variável soroproteção como variável dependente, as variáveis independentes idade, presença de AR e menor MGT pré-vacinal se associaram com ausência de soroproteção quando os grupos de pacientes com AR e controles foram avaliados juntos.

Quando apenas os pacientes com AR foram avaliados, as análises multivariadas mostraram que a maior idade, o uso de metotrexato, de abatacepte, menores valores de DAS28 e menor MGT prévia foram associadas com ausência de soroproteção ($p<0,05$), mas não o número de DMARDs ou a presença de atividade clínica ($p>0,05$).

Quando a variável dependente utilizada foi a soroconversão, as variáveis independentes idade e presença de AR se associaram com a falha de resposta quando os grupos de pacientes com AR e controles foram avaliados juntos.

Quando apenas os pacientes com AR foram avaliados, as análises multivariadas mostraram que maior idade, uso de metotrexato e menores valores de DAS28 foram associadas com ausência de soroconversão ($p<0,05$), mas não o uso de abatacepte, o número de DMARDs ou a presença de atividade clínica ($p>0,05$). A probabilidade de redução na soroconversão dos pacientes em uso de metotrexato foi de 49% (OR 0,51; IC 95%: 0,32 a 0,82; $p=0,005$).

4.6 Avaliação de doença após a imunização

Quase todos os pacientes com AR (97,4%) responderam “não” à questão sobre alteração da atividade de doença provocada pela vacinação, ou seja, não perceberam alterações provocadas pela vacina. Todos os que responderam “sim” (2,6%) relataram piora clínica. De fato, o DAS28 desse grupo específico piorou significativamente em todos os componentes (Tabela 6), mas nenhuma variável no momento zero foi preditora dessa piora. A maioria dos parâmetros clínicos e laboratoriais de atividade de doença não teve alterações significantes no grupo inteiro, a não ser por uma discreta queda no DAS28 e no número de articulações com dor (Tabela 6).

4.7 Eventos adversos

A vacina foi bem tolerada: não foram observados eventos adversos graves em nenhum dos grupos durante o seguimento de 3 semanas. Nenhum paciente solicitou consultas extras, embora tenham reportado significantemente mais efeitos adversos que os controles (140 eventos/100 pacientes vs. 87/100 controles; $p<0,005$).

Reações locais foram mais frequentemente observadas nos controles ($p=0,032$) enquanto reações sistêmicas leves foram mais observadas em pacientes com AR ($p=0,01$) (Tabela 7). Sintomas locais prevaleceram durante os primeiros dias (88%), enquanto os sintomas sistêmicos se concentraram por volta dos primeiros 15 dias (73%) após a vacinação.

Tabela 6 - Dados demográficos, parâmetros da doença e tratamento de acordo com o relato de piora clínica em pacientes com AR após a vacinação contra influenza A/H1N1

n(%)	Total n=340		Sem piora n=331 (97,4)		Com piora n=9 (2,6)	
	Antes	**Após**	**Antes**	**Após**	**Antes**	**Após**
Dados demográficos						
Sexo feminino, n (%)	295 (86,8)		287 (86,7)		8 (88,9)	
Idade atual, anos	55,8 (11,5)		55,8 (11,4)		56,6 (15)	
Tempo de doença, anos	16,7 (10,4)		16,7 (10,5)		16,9 (8,9)	
Positividade do FR, n (%)	249 (72,8)		242 (73,1)		7 (77,7)	
Atividade de doença						
DAS28	3,66 (1,35)	3,49 (1,36)*	3,67 (1,37)	3,51 (1,39)†	3,71 (1,23)	5,11 (1,57)#
Nº articulações com dor	3,7 (4,8)	3,7 (5,4)	3,9 (5,2)	3,8 (5,7)	3,8 (3,9)	9,8 (10,1)#
Nº articulações com edema	4,4 (4,6)	3 (3,9)*	4,4 (4,7)	3,1 (4)†	4,3 (3,6)	7,9 (5,1)#
VHS, mm/1ª hora	20,7 (20,2)	20,6 (20,2)	20,5 (20,2)	20,5 (20,1)	18,3 (10,9)	30,2 (17,1)#
EVA global do paciente, 0-100 mm	37,9 (26,6)	36,9 (27,4)	38,5 (26,7)	37,1 (27,3)	30,2 (24,1)	55,4 (28,8)#
EVA de dor do paciente, 0-100 mm	38,3 (27,0)	37,5 (28,2)	38,3 (27)	37,4 (28,2)	40,6 (31,1)	51 (28,3)#
Tratamento						
Prednisona, n(%)	247 (72,6)		240 (72,3)		7 (77,8)	
Dose de prednisona, mg/d	8,6 (5,2)		8,5 (5,2)		7,9 (3,9)	
Metotrexato (MTX), n(%)	215 (63,2)		211 (63,7)		4 (44,4)	
Dose de MTX, mg/semana	19,2 (5,6)		19,2 (5,6)		19,4 (4,3)	
Leflunomida (20mg/d), n(%)	146 (42,9)		141 (42,6)		5 (55,5)	
Cloroquina, n(%)	124 (36,5)		120 (36,3)		4 (44,4)	
Agentes anti-TNF, n(%)	47 (13,8)		46 (13,9)		1 (11,1)	

Valores expressos em número (%) ou média (DP)

AR = artrite reumatoide; FR = fator reumatoide; DAS28 = *disease activity score*; VHS = velocidade de hemossedimentação; PCR = proteína C reativa; EVA = escala visual analógica; MTX = metotrexato; anti-TNF = anti-*tumoral necrosis factor*. Não foram observadas diferenças entre as características

demográficas, atividade de doença ou tratamento entre os 3 grupos (p>0,05).

* † # p<0,05 = comparação entre os valores antes e depois da vacina no total de pacientes com AR, nos pacientes que não referiram piora e nos pacientes que referiram piora, respectivamente; medicações prescritas a menos de 10% dos pacientes não foram incluídas.

Tabela 7 - Eventos adversos da vacinação contra influenza pandêmica A/H1N1 em pacientes com AR e controles

	AR n=336	Controles n=234	p
Reações locais, n(%)	30 (8,9)	35 (15,0)	0,032
Febre/calafrios, n(%)	39 (11,6)	10 (4,3)	0,0021
Artralgia/mialgia, n(%)	92 (27,4)	25 (10,7)	0,0001
Sintomas gripais, n(%)	73 (21,7)	30 (12,8)	0,0077

Valores expressos em número (%).
AR = artrite reumatoide. Quatro pacientes com AR tinham diários de efeitos adversos incompletos e foram excluídos da análise. Reações locais = presença de um ou mais dos seguintes no local de aplicação da vacina: dor, eritema, edema ou prurido. Sintomas gripais = presença de um ou mais dos seguintes sintomas: dor de garganta, tosse, rinorreia ou congestão nasal.

5 DISCUSSÃO

Esse é o maior estudo avaliando a vacina sem adjuvante contra a influenza pandêmica A/H1N1 em pacientes com AR submetidos a tratamento rotineiro com imunossupressores e demostrou uma redução na resposta vacinal nesses indivíduos.

Embora houvesse predomínio de mulheres entre os pacientes (7 mulheres para cada homem, como esperado para a população brasileira acometida por AR)[11], o gênero não parece ter sido a causa da menor imunogenicidade, inclusive porque mulheres sabidamente atingem maiores títulos de anticorpos contra bactérias e vírus[12].

A idade é um reconhecido fator que pode alterar a resposta vacinal. Entretanto, a imunogenicidade reduzida à vacina foi observada mesmo quando uma subanálise dos grupos de pacientes e controles com idades comparáveis foi realizada, como já descrito para outras doenças reumáticas autoimunes[7,13]. Além disso, após análises multivariadas usando a soroproteção e a soroconversão, os desfechos principais, como variáveis dependentes, a AR foi independentemente associada a uma resposta imune prejudicada.

A redução da resposta sorológica poderia estar relacionada ao estado imunológico alterado da AR, à atividade da doença, à medicação ou à baixa imunogenicidade da vacina. Em relação à atividade de doença,

trabalhos prévios sugerem que esse fator parece não interferir na resposta vacinal em pacientes com AR[14]. Entretanto, foi demonstrada correlação entre a magnitude da resposta imune a *Escherichia coli* e atividade de doença em pacientes com AIJ[15]. De fato, observamos menores valores de DAS28 entre os não respondedores comparados aos respondedores, porém com médias muito próximas e dentro da mesma faixa de atividade, limitando o significado clínico desse achado. Além disso, a presença de atividade clínica (DAS>2,6) não se manteve associada à resposta vacinal após as análises multivariadas, reforçando que ela não prejudica a imunogenicidade desta vacina. A atividade de doença também não foi relacionada a efeitos adversos, um fato relevante para futuras recomendações de protocolos de vacinação[1].

Como a maioria dos pacientes estava em terapia combinada, não foi possível avaliar perfeitamente os efeitos da doença por si mesma nem das medicações independentemente. Entretanto, o metotrexato nas doses observadas em nossos pacientes [média(DP) 19,2(5,6) mg/semana] foi relacionado a respostas sorológicas reduzidas em todos os desfechos. Um estudo recente com a vacina com adjuvante contra a influenza pandêmica A/H1N1 também ligou o metotrexato a baixas respostas anticórpicas à vacina[13]. Já estudos com a vacina trivalente sazonal falharam em mostrar essa associação[14,16-17], mas as doses de metotrexato descritas nesses casos eram menores[14,17], o que pode justificar a discrepância.

Não observamos efeito deletério do uso de corticoide ou de DMARDs tradicionais sobre a resposta vacinal. Diferentemente, outro estudo

com a vacina com adjuvante associou leflunomida e outros imunossupressores com baixa MGT pós-vacinal[13]. Entretanto, esse é um desfecho secundário, com valor limitado. Além disso, em corroboração ao nosso achado, estudos prévios com a vacina contra influenza sazonal falharam em mostrar influência consistente do uso de DMARDs clássicos ou corticoides[14,17,18]. De qualquer forma, a dose de corticoide usada pelos pacientes no presente estudo, em média(DP) 8,6(5,2) mg/dia, pode não ser suficiente para alterar a resposta vacinal.

Em relação às DMARDs biológicas, as respostas foram variáveis. O uso de abatacepte apresentou-se deletério à resposta humoral contra a vacina da influenza pandêmica A/H1N1 em pacientes com AR, com total ausência de soroconversão. Esse achado é corroborado por um estudo com vacina pandêmica com adjuvante em uma população mista de AR e espondiloartrites, em que apenas 35% dos pacientes sorocoverteram[19], apontando a inibição da coestimulação dos linfócitos T como possivelmente associada ao prejuízo da resposta imunológica humoral.

Já o rituximabe, em nosso estudo, provocou redução significante apenas nos desfechos secundários (MGT pós-vacinal e FI-MGT), diferentemente do que é descrito em estudos com influenza sazonal[20,21] e pandêmica com adjuvante[13,19], em que o rituximabe prejudicou intensamente a resposta vacinal. Por outro lado, o tocilizumabe não alterou nenhum dos desfechos em nosso estudo. Entretanto, o baixo número de pacientes em uso de cada um desses biológicos limita uma análise mais precisa.

Em relação aos agentes bloqueadores do TNF, eles não prejudicaram a resposta imune, de forma semelhante a outros estudos usando a vacina contra influenza pandêmica A/H1N1 com adjuvante[13,19]. Nos estudos com influenza sazonal, de uma forma geral, os pacientes em uso de anti-TNF tiveram taxas de soroproteção adequadas ou apenas ligeiramente reduzidas[14,17,18,22,23].

Nossos achados também ajudaram a esclarecer a controvérsia sobre o uso de vacina com ou sem adjuvante, pois nossos resultados foram similares àqueles encontrados em outro estudo usando uma dose única da vacina contra influenza pandênica A/H1N1 com adjuvante em 82 pacientes com AR[13]. Além disso, quando diferentes preparações da vacina foram testadas em um grande estudo na população chinesa, a formulação sem adjuvante foi mais efetiva com a mesma carga antigênica[24].

Os dados claramente demostram a segurança em curto prazo da vacina contra influenza pandêmica A/H1N1 sem adjuvante em pacientes com AR. As provas inflamatórias permaneceram inalteradas e a piora da doença, referida por 9 (2,6%) dos 340 pacientes não pode ser totalmente atribuída à vacinação, pois não pode ser diferenciada de exacerbações naturais da doença. A discreta queda do DAS28 e do número de articulações com edema após a vacinação foi abaixo do valor clínico significante[9.] Além disso, nossos pacientes estavam sob tratamento regular objetivando o controle da doença, não sendo pré-selecionados de acordo com o uso de medicação ou duração do tratamento, visto que o estudo foi feito durante a campanha nacional de vacinação contra influenza A/H1N1[5.]

Baixas taxas de soroconversão e soroproteção em pacientes com AR, particularmente naqueles em uso de metotrexato e abatacepte, têm grandes implicações clínicas e sugerem que inúmeros pacientes imunossuprimidos podem estar em risco infeccioso, a despeito da vacinação. Junto ao achado de melhora da resposta vacinal a uma segunda dose da vacina com adjuvante contra a influenza pandêmica A/H1N1[13] e da resposta à influenza sazonal em pacientes com AR, esse estudo sinaliza a necessidade de estratégias vacinais específicas para cada vacina nova nessa população.

Em conclusão, o presente estudo prospectivo da vacinação sem adjuvante contra influenza pandêmica A/H1N1 em pacientes com AR demostra redução da imunogenicidade, sobretudo em pacientes em uso de metotrexato e abatacepte, mas sem efeitos colaterais graves e sem prejuízo em curto prazo do controle de atividade da própria doença.

6 CONCLUSÃO

a) A imunogenicidade da vacina sem adjuvante contra a influenza pandêmica A/H1N1 foi diminuída em pacientes com AR.

b) Os fatores que influenciaram negativamente a imunogenicidade entre os pacientes com AR foram a idade e o uso de metotrexato e de abatacepte.

c) A vacinação sem adjuvante contra a influenza pandêmica A/H1N1 em pacientes com AR mostrou-se segura em curto prazo, tanto em termos gerais como sobre o controle da atividade da doença.

7 ANEXOS

Anexo A - Aprovação Cappesq do Projeto de Pesquisa nº 0114/10

HOSPITAL DAS CLÍNICAS
DA FACULDADE DE MEDICINA
DA UNIVERSIDADE DE SÃO PAULO

APROVAÇÃO

A Comissão de Ética para Análise de Projetos de Pesquisa - CAPPesq da Diretoria Clínica do Hospital das Clínicas e da Faculdade de Medicina da Universidade de São Paulo, em sessão de 10/03/2010, **APROVOU** o Protocolo de Pesquisa nº **0114/10**, intitulado: "**RESPOSTA À VACINA ANTI-H1N1 EM PACIENTES IMUNOSSUPRIMIDOS COM DOENÇAS REUMÁTICAS**" apresentado pelo Departamento de **CLÍNICA MÉDICA**, inclusive o Termo de Consentimento Livre e Esclarecido.

Cabe ao pesquisador elaborar e apresentar à CAPPesq, os relatórios parciais e final sobre a pesquisa (Resolução do Conselho Nacional de Saúde nº 196, de 10/10/1996, inciso IX.2, letra "c").

Pesquisador (a) Responsável: **Profa. Dra. Eloisa Silva Dutra de Oliveira Bonfá**
Pesquisador (es) Executante(s): **Nádia Emi Aikawa, Ivan Leonardo Avelino França e Silva, Alexander R. Precioso, Jozélio Freire de Carvalho, Alberto José da Silva Duarte, Ana Cristina de Medeiros, Julio Cesar Bertacini de Moares, Carla Gonçalves, Lucia Maria de Arruda Campos, Clovis Artur Almeida da Silva, Adriana Maluf Elias Sallum, Adriana Almeida de Jesus.**

CAPPesq, 11 de Março de 2010

Prof. Dr. Eduardo Massad
Presidente da Comissão de Ética para Análise de Projetos de Pesquisa

Comissão de Ética para Análise de Projetos de Pesquisa do HCFMUSP e da FMUSP Diretoria Clínica do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo Rua Ovídio Pires de Campos, 225, 5º andar - CEP 05403 010 - São Paulo – SP Fone: 011 3069 6442 Fax: 011 3069 6492 e-mail: cappesq@hcnet.usp.br / secretariacappesq2@hcnet.usp.br

Anexo B - Termo de Consentimento Livre e Esclarecido

Anexo I

HOSPITAL DAS CLÍNICAS

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TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

(Instruções para preenchimento no verso)

I - DADOS DE IDENTIFICAÇÃO DO SUJEITO DA PESQUISA OU RESPONSÁVEL LEGAL

1.NOME DO PARTICIPANTE.:..... .....

DOCUMENTO DE IDENTIDADE Nº : ..... SEXO : .M ☐ F ☐

DATA NASCIMENTO: ...../...../.....

ENDEREÇO ..... Nº ..... APTO: .....

BAIRRO: ..... CIDADE .....

CEP:..... TELEFONE: DDD (.....) .....

2.RESPONSÁVEL LEGAL.....

NATUREZA (grau de parentesco, tutor, curador etc.) .....

DOCUMENTO DE IDENTIDADE :.....SEXO: M ☐ F ☐

DATA NASCIMENTO.: ...../...../.....

ENDEREÇO: ..... Nº ..... APTO: .....

BAIRRO: ..... CIDADE: .....

CEP: ..... TELEFONE: DDD (.....).....

II - DADOS SOBRE A PESQUISA CIENTÍFICA

1. TÍTULO DO PROTOCOLO DE PESQUISA**:** RESPOSTA À VACINA CONTRA INFLUENZA A(H1N1)2009 EM PACIENTES COM DOENÇAS REUMÁTICAS AUTOIMUNES

PESQUISADOR: Profa. Dra. Eloísa Silva Dutra de Oliveira Bonfá

CARGO/FUNÇÃO: Professor Titular

INSCRIÇÃO CONSELHO REGIONAL Nº 42708

UNIDADE DO HCFMUSP: Clínica Médica – Disciplina de Reumatologia

3. AVALIAÇÃO DO RISCO DA PESQUISA:

SEM RISCO ☐ RISCO MÍNIMO ☐ RISCO MÉDIO **X**

RISCO BAIXO ☐ RISCO MAIOR ☐

(probabilidade de que o indivíduo sofra algum dano como consequência imediata ou tardia do estudo)

4.DURAÇÃO DA PESQUISA: total 12 meses

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III - REGISTRO DAS EXPLICAÇÕES DO PESQUISADOR AO PACIENTE, CONTROLE OU SEU REPRESENTANTE LEGAL SOBRE A PESQUISA CONSIGNANDO:

1. Justificativa e objetivos da pesquisa – Estamos convidando você para participar de um protocolo de pesquisa, que envolve pacientes com doenças reumáticas. Por causa dos remédios que você toma e da sua doença, é indicado que você receba vacinas contra doenças que podem ser mais comuns e mais graves em pessoas tomando esses medicamentos. Assim, é indicado que você receba vacina contra o vírus da gripe (influenza), todos os anos. Esse vírus causa infecções de nariz, garganta, ouvido e pulmão (pneumonia), que podem ser doenças graves, com risco de vida. Em virtude da pandemia de "gripe suína" em 2009, a vacina contra este vírus (influenza A(H1N1) também está indicada para pacientes como você, com o objetivo de diminuir complicações e a mortalidade pelo vírus.

Vacinação de funcionários: é indicado que você receba vacina contra o vírus da gripe (influenza), todos os anos. Esse vírus causa infecções de nariz, garganta, ouvido e pulmão (pneumonia), que podem ser doenças graves, com risco de vida. Em virtude da pandemia de "gripe suína" em 2009, a vacina contra este vírus (influenza A(H1N1) também está indicada para indivíduos como você, com o objetivo de diminuir complicações e a mortalidade pelo vírus.

2. Procedimentos que serão utilizados e propostos. O médico responsável pelo seu tratamento continuará sendo o mesmo. Ele indicará exames, orientará o tratamento e o acompanhará em caso de complicações. Caso você concorde em participar desse protocolo será realizado um exame físico e serão feitos questionários sobre o seu estado de doença. Além disso, serão colhidos exames de sangue em 2 momentos: antes de receber a vacina e 21 dias após. Esses exames necessitarão da coleta de um pouco de sangue (20mL – menos que uma xícara de café). Eles não serão utilizados para acompanhamento de efeitos colaterais individuais, ou seja, de efeitos desagradáveis ou problemas que possam surgir com o tratamento. Serão colhidos somente para fins de estudo. Parte de sangue colhido será usada para estudar a resposta do corpo a essa vacina, para ver se os remédios que você utiliza interferem ou não na capacidade do corpo de se defender contra esse germe. Essa resposta é medida através da dosagem de anticorpos, que são substâncias produzidas pelas células de defesa do corpo. O restante do sangue será utilizado para avaliar a atividade da sua doença. Caso você apresente sintomas de infecção respiratória (resfriado, gripe, sinusite) durante o tempo do estudo deverá comunicar o seu médico. Não há procedimentos, exames ou remédios experimentais. Lembre-se de que todos os exames que forem feitos neste estudo são para fins de pesquisa. Os exames e o acompanhamento da doença individualmente serão feitos e checados pelo seu médico.

Vacinação de funcionários: Caso você concorde em participar desse protocolo será realizado um exame físico e serão feitos questionários sobre o seu estado de saúde. Além disso, serão colhidos exames de sangue em 2 momentos: antes de receber a vacina e 21 dias após. Esses exames necessitarão da coleta de um pouco de sangue (10mL – menos que uma xícara de café). Serão colhidos somente para estudar a resposta do corpo a essa vacina. Essa resposta é medida através da dosagem de anticorpos, que são substâncias produzidas pelas células de defesa do corpo. Caso você apresente sintomas de infecção respiratória (resfriado, gripe, sinusite) durante o tempo do estudo deverá comunicar o seu médico. Não há procedimentos, exames ou remédios experimentais. Lembre-se de que todos os exames que forem feitos neste estudo são para fins de pesquisa.

3. Desconfortos e riscos esperados – desconforto leve relativo à necessidade coleta de sangue por punção venosa, ou seja, risco de formar um hematoma ou mancha roxa no lugar da picada para retirada de pequena quantidade de sangue para os exames. Riscos inerentes à vacinação: coceira, dor no local, vermelhidão, endurecimento no local da vacina, febre, calafrios, dor de cabeça, dores no corpo e nas juntas e diarréia.

4. Procedimentos alternativos que possam ser vantajosos para o indivíduo – não se aplica.

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IV - ESCLARECIMENTOS DADOS PELO PESQUISADOR SOBRE GARANTIAS DO SUJEITO DA PESQUISA CONSIGNANDO:

1. acesso, a qualquer tempo, às informações sobre procedimentos, riscos e benefícios relacionados à pesquisa, inclusive para dirimir eventuais dúvidas. Você terá acesso a qualquer momento a todas informações relacionadas ao estudo, que poderão também ser relatadas ao seu médico. Os médicos do estudo estarão disponíveis para responder a suas dúvidas.

2. liberdade de retirar seu consentimento a qualquer momento e de deixar de participar do estudo, sem que isto traga prejuízo à continuidade da assistência. Sua participação neste estudo é voluntária. A qualquer momento seu consentimento poderá ser retirado. Não haverá qualquer prejuízo para o seu tratamento aqui no hospital. Você continuará a receber o tratamento, mesmo que não queira participar do protocolo de estudo. Se decidir sair do estudo não precisa declarar o motivo.

3. salvaguarda da confidencialidade, sigilo e privacidade. Todos os dados referentes a você e seu tratamento são confidenciais, somente seu médico e os membros do estudo sabem que você estará participando. Se o estudo for publicado, você não será identificado pelo nome.

4. disponibilidade de assistência no HCFMUSP, por eventuais danos à saúde, decorrentes da pesquisa. Os recursos do Hospital das Clínicas estarão disponíveis para qualquer assintência a sua saúde por problemas relacionados à pesquisa, devendo entrar em contato com o Serviço de Reumatologia do Hospital das Clínicas. Entretanto, a responsabilidade de acompanhamento e tratamento dos efeitos colaterais inerentes ao uso da(s) medicação(ões) não observados durante o tempo em que você estiver no estudo, será do seu médico.

5. viabilidade de indenização por eventuais danos à saúde decorrentes da pesquisa. Não se aplica. A vacinação das pessoas que vão ficar com as defesas do corpo diminuídas por causa dos remédios e pela doença é recomendada pelo Ministério da Saúde.

V. INFORMAÇÕES DE NOMES, ENDEREÇOS E TELEFONES DOS RESPONSÁVEIS PELO ACOMPANHAMENTO DA PESQUISA, PARA CONTATO EM CASO DE INTERCORRÊNCIAS CLÍNICAS E REAÇÕES ADVERSAS.

Dra. Eloisa Silva Dutra de Oliveira Bonfá - 3061-7492, 3061-7490, Dr. Jozélio Freire de Carvalho, Dr. Clóvis Artur Almeida da Silva, Dr, Ivan Leonardo Avenino França e Silva, Ana Cristina de Medeiros, Carla Gonçalves, Julio César Bertacini de Moraes, Dra Nádia Emi Aikawa, Dra Lucia Maria Arruda Campos – **CEDMAC**: **3069-8023, 3069-8024, Secretaria da reumatologia :3061-7492, 3061-7490, Instituto da Criança: 30698510, 30698563.**

Em caso de questões quanto à ética da pesquisa ou dos seus direitos como paciente, favor dirigir-se à CAPPESQ. Tel:3069-6642

Em casa de questões quanto à ética da pesquisa ou dos seus direitos como paciente, favor dirigir-se à CAPPESQ. Tel:3069-6642

Assinatura do paciente/representante legal Data ____/____/____

Assinatura da testemunha Data ____/____/____

para casos de pacientes menores de 18 anos, analfabetos, semi-analfabetos ou portadores de deficiência auditiva ou visual.

(Somente para o responsável do projeto)

Declaro que obtive de forma apropriada e voluntária o Consentimento Livre e Esclarecido deste paciente ou representante legal para a participação neste estudo.

Assinatura do responsável pelo estudo Data ____/____/____

Anexo C – Publicação do trabalho da tese

Reduced seroprotection after pandemic H1N1 influenza adjuvant-free vaccination in patients with rheumatoid arthritis: implications for clinical practice

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► Additional supplementary tables and figure are published online only. To view these files please visit the journal online at (http://ard.bmj.com)

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ABSTRACT

Background Reduced response to pandemic (2009) H1N1 (pH1N1) vaccine in patients with rheumatoid arthritis (RA) was recently reported.

Objectives To evaluate the contribution of age, disease activity, medication and previous antibody levels to this reduced response.

Methods 340 adult RA patients and 234 healthy controls were assessed before and 21 days after adjuvant-free influenza A/California/7/2009 (pH1N1) vaccine. Disease activity (DAS28), current treatment and pH1N1 antibody titres were collected. Seroprotection, seroconversion and factor increase in geometric mean titre (GMT) were calculated and adverse events registered.

Results RA and controls showed similar ($p>0.05$) prevaccination GMT (8.0 vs 9.3) and seroprotection (10.8% vs 11.5%). After vaccination a significant reduction ($p<0.001$) was observed in all endpoints: GMT and factor increase in GMT, seroprotection and seroconversion rates. Disease activity did not preclude seroconversion or seroprotection and remained unchanged in 97.4% of patients. Methotrexate was the only disease-modifying antirheumatic drug associated with reduced responses ($p=0.001$). Vaccination was well tolerated.

Conclusions The data confirmed both short-term anti-pH1N1 vaccine safety and, different from most studies with seasonal influenza, reduced seroprotection in RA patients, unrelated to disease activity and to most medications (except methotrexate). Extrapolation of immune responses from one vaccine to another may therefore not be possible and specific immunisation strategies (possibly booster) may be needed. Clinicaltrials.gov no NCT01151644.

The benefits of vaccination in avoiding vaccine-preventable diseases in patients with immune-inflammatory diseases such as rheumatoid arthritis (RA) are accepted.[1–3] Nevertheless, there is concern regarding vaccine safety and efficacy.[2] This is particularly important for emergent pandemic diseases such as pandemic (2009) H1N1 (pH1N1) influenza.

After the pH1N1A/California/7/2009 pandemic,[4] inactivated monovalent vaccines became available through national programmes.[5] Vaccination dosing schedules were developed in healthy individuals.[6] We recently reported, in a large cohort of autoimmune rheumatic diseases including 343 RA patients, the overall short-term safety but reduced immunogenicity of a new adjuvant-free influenza A p/H1N1 vaccine,[7] without addressing correlation with RA activity and medication. Our study assessed the relationship between reduced response to adjuvant-free pH1N1 vaccine and both RA disease activity and treatment with classic and biological disease-modifying antirheumatic drugs (DMARD).

METHODS

Study design and participants

This prospective single-centre study was conducted during the public national health influenza A pH1N1(2009) vaccination campaign in Brazil. It was approved by the Institutional Review Board (clinicaltrials.gov no NCT01151644) and included two stages, vaccination (from 22 March to 2 April) and a 21-day follow-up.

RA (1987 American College of Rheumatology criteria)[8] patients in regular follow-up at the outpatient clinics were recruited through invitation letters. Out of 562 invited patients, 391 (69.6%) received the vaccine and 349 (89.3%) completed the two phases; 340 (87.0%) patients were included in this study (complete serology, clinical and therapeutic data). Healthy control individuals were recruited from the hospital's immunisation centre. Among 326 vaccinated controls, 234 (71.8%) completed the study.

Inclusion and exclusion criteria

Inclusion and exclusion criteria are available in supplementary table S1 (available online only) and were similar to those previously described.[7]

Vaccine

The vaccine, Sanofi Pasteur Influenza A (H1N1) 2009 was a novel monovalent adjuvant-free vaccine (A/California/7/2009/Butantan Institute/Sanofi Pasteur, São Paulo, Brazil). The active component was a split inactivated influenza virus containing 15 µg haemagglutinin of influenza A/California/07/2009 (pH1N1) virus-like strain (NYMCx-179A) per 0.5-ml dose.[7] It was available as 5-ml multidose vials with thimerosal (45 µg/0.5-ml dose) added as preservative.

Study procedures

All subjects were vaccinated with a single intramuscular dose of pH1N1 vaccine. Disease

activity (disease activity score in 28 joints (DAS28)–erythrocyte sedimentation rate (ESR))[9] evaluation and blood sample collection were performed before and 21 days after the vaccination.

Safety assessments

A 21-day symptom diary card for prospective completion was given to each participant following vaccination and was returned 21 days later. All new symptoms, recorded or not in the diary, were reviewed by the investigators and causal relation with the vaccine was assessed. All RA patients answered one specific (yes or no) question about their perception of vaccine interference on disease activity responses.

Laboratory assays

The immunogenicity of pH1N1 A/California/7/2009-like virus vaccine was evaluated (haemagglutination inhibition assay) at Adolfo Lutz Institute.[7 10] Antibody titres were assessed at baseline and 21 days post-immunisation. Geometric mean titres (GMT) were calculated. Serological endpoints were evaluated: seroprotection rate defined as the percentage of patients with titre 1:40 and seroconversion rate as the percentage of patients with a fourfold or greater increase in vaccination titre if prevaccination titre was 1:10 or greater, or postvaccination titre was 1:40 or greater if prevaccination titre was less than 1/10. The factor increase in GMT was also calculated. ESR and CRP were assessed.

Statistical analysis

Two-sided 95% CI were calculated assuming binomial distributions for dichotomous variables (Clopper–Pearson method) and log normal distribution for haemagglutination inhibition titres. Categorical variables were compared by Fisher's exact test or the Fisher–Freeman–Halton exact test; normally or non-normally distributed variables were compared using the t test or Wilcoxon rank-sum test. A multiple logistic regression model was applied to analyse interaction between demographic characteristics, medications and seroconversion. All tests were two-sided, with a 0.05 significance level.

RESULTS

Demographic characteristics

Age (55.8±11.5 vs 36.63±12.5 years, p<0.0001) and female predominance (86.7% vs 66.8%, p<0.0001) were significantly higher in RA patients compared with controls. Clinical data are depicted in table 1.

Disease activity modifications due to immunisation

Nearly all (97.4%) RA patients answering one specific question about changes in disease activity after immunisation reported no alterations. All nine (2.6%) patients describing some change related worsening of symptoms. In fact, their DAS28 increased due to a significant rise in all components, with no baseline variable able to single out this small patient group (see supplementary table S2, available online only).

Clinical and laboratory parameters of disease activity remained unchanged in the group as a whole, except for slight non-clinically significant decreases in DAS28 and swollen joint number (table 1).

Pre-existent antibodies to pH1N1 (2009) and response to immunisation

Prevaccination seroprotection and GMT were similar in both groups (p>0.05; table 2). Conversely, following vaccination, RA patients showed reduced seroprotection and seroconversion rates compared with controls. Despite a significant increase in postvaccination GMT in both groups, GMT and factor increase in GMT were significantly lower in RA patients compared with controls (table 2).

Due to age differences, subanalysis was performed comparing 88 RA patients and 184 age-matched controls (mean ages 40.8 years (39.4 to 42.1) vs 40.8 years (39.5 to 42.1); p>0.05). There were comparable (p>0.05) prevaccination seroprotection rates (8.0% (2.3 to 13.6); 9.8% (5.5 to 14.1)) and GMT (8.2 (6.9 to 9.8); 9.0 (7.9 to 10.2)) and lower postvaccination seroprotection rates (67.0% (57.2 to 76.9); 83.2% (77.8 to 88.6); p=0.04) and seroconversion rates (63.6% (53.5 to 73.7); 76.1% (69.9 to 82.3); p=0.005) in RA patients. Postvaccination GMT in this RA subgroup (83.9 (60.3 to 116.7); 116.6 (96.2 to 141.3)) and factor increase in GMT (10.2 (7.5 to 13.9); 13.0 (10.7 to 15.8)) values were non-statistically significantly lower. Data on age subsets are shown in supplementary table S3 and figure S1, available online only.

Influence of disease characteristics and medication

High disease activity levels (14.5% of patients) did not preclude immune response (figure 1).

Patients were in regular follow-up receiving traditional and biological DMARD, usually in combination. Pre and postvaccination GMT and seroprotection rates, factor increase in GMT and seroconversion rates in patients with or without medications are depicted in table 2 and compared with controls.

Anti-tumour necrosis factor (TNF) agents were grouped together due to small patient numbers: 20, 16 and 11 on infliximab, adalimumab and etanercept, respectively. Only 11 (3.2%)

Table 1 Demography, treatment schedules and disease activity parameters before and after vaccination in RA patients

	RA patients before vaccine	RA patients after vaccine
n (%)	340(100)	–
Female n (%)	295 (86.8)	–
RF positivity, n (%)	249 (72.8)	
Age, years	55.8 (11.5)	–
Disease duration, years	16.7 (10.4)	–
DAS28–ESR	3.66 (1.35)	3.49 (1.36)*
No of tender joints	3.7(4.8)	3.7 (5.4)
No of swollen joints	4.4 (4.6)	3.0 (3.87)*
ESR, mm/h	20.7 (20.2)	20.6 (20.2)
CRP, mg/dl	12.1 (18.2)	12.7 (18.0)
PGHA, 0–100 mm	37.9 (26.6)	36.9 (27.4)
Pain, VAS 0–100 mm	38.3 (27.0)	37.5 (28.2)
Treatment		
Corticosteroids, n (%)	247 (72.6)	–
Dose of corticosteroids, mg/day	8.6 (5.2)	–
Methotrexate, n (%)	215 (63.2)	–
Methotrexate, mg/week	19.2 (5.6)	–
Leflunomide, 20 mg/day, n (%)	146 (42.9)	–
Chloroquine, n (%)	124 (36.5)	–
Anti-TNF agents, n (%)	47 (13.8)	–

Data are expressed as mean (SD) unless otherwise specified.
Anti-TNF agents, adalimumab, infliximab, etanercept; medications prescribed to less than 10% of the patients were not included.
*p <0.05.
CRP, C-reactive protein; DAS28, 28-joint disease activity score; ESR, erythrocyte sedimentation rate; PGHA, patient global health assessment; RA, rheumatoid arthritis; RF, rheumatoid factor; TNF, tumour necrosis factor; VAS, visual analogue scale.

Table 2 Serological data before and after influenza a pandemic (pH1N1) 2009 vaccine in controls and RA patients (total population, RF and medication subsets)

	Prevaccination		Posvaccination			
Subset	**GMT**	**Seroprotection**	**GMT**	**Seroprotection**	**Factor increase**	**Seroconversion**
Controls(234)	9.3(8.2 to 10.5)	11.5(7.4 to 15.6)	122.9(103.5 to 146.0)	82.9(77.5 to 87.5)	13.2(11.1 to 15.8)	76.9(71.0 to 82.2)
RA group(340)	8.0(7.3 to 8.8)	10.8(7.5 to 14.1)	57.5(48.0 to 68.9)*	60.0(54.5 to 65.3)*	7.2(6.1 to 8.5)*	53.2(47.8 to 58.6)*
RF (340)						
No (91)	7.8(6.5 to 9.4)	9.6(3.3 to 16.0)	48.1(33.2 to 69.6)*	54.2(43.4 to 65.0)*	6.2(4.4 to 8.7)*	48.2(37.4 to 59.0)*
Yes (249)	7.9(7.1 to 8.8)*	10.0(6.3 to 13.8)	59.6(48.2 to 73.6)*	64.5(55.4 to 67.5)*	7.5(6.2 to 9.2)*	54.2(48.0 to 60.4)*
Corticosteroids						
No (93)	7.8(7.0 to 8.6)*	10.1(6.4 to 13.9)	58.4(47.1 to 72.5)*	60.3(54.2 to 66.4)*	7.5(6.2 to 9.2)*	54.7(48.4 to 60.9)*
Yes (247)	8.6(7.2 to 10.4)	12.9(6.1 to 19.7)	55.1(39.4 to 77.0)*	59.1(49.0 to 69.2)*	6.4(4.7 to 8.7)*	49.5(39.2 to 59.7)*
Methotrexate						
No (125)	8.0(6.9 to 9.2)	12.9(7.0 to 12.8)	90.9(66.3 to 124.9) †	71.8(63.8 to 79.7)*†	11.4(8.4 to 15.4) †	65.3(56.9 to 73.7) †
Yes (215)	8.0(7.1 to 9.0)	9.7(5.8 to 13.7)	44.2(35.7 to 54.7)*†	53.2(46.6 to 59.9)*†	5.5(4.6 to 6.7)*†	46.3(39.6 to 53.0)*†
Chloroquine						
No (216)	7.6(6.8 to 8.5)	10.1(6.1 to 14.2)	57.4(45.4 to 72.6)*	60.8(54.3 to 67.3)*	7.5(6.1 to 9.3)*	55.3(48.7 to 61.9)*
Yes (124)	8.7(7.5 to 10.1)	12.2(6.4 to 18.0)	57.7(43.5 to 76.5)*	58.5(49.8 to 67.3)*	6.6(5.1 to 8.7)*	49.6(40.7 to 58.5)*
Leflunomide						
No (194)	8.8(7.7 to 9.9)	12.9(8.2 to 17.6)	49.2(39.1 to 61.9)*	56.7(49.7 to 63.7)*	5.6(4.6 to 6.8)*	50.5(43.5 to 57.6)*
Yes (146)	7.1(6.3 to 7.9)*	8.3(3.8 to 12.7)	71.3(53.5 to 95.1)*	64.8(57.0 to 72.6)*	10.1(7.6 to 13.4)*	57.2(49.1 to 65.3)*
Anti-TNF						
No (293)	8.1(7.2 to 8.9)	11.2(7.4 to 15.0)	55.5(45.1 to 68.3)*	58.8(52.9 to 64.8)*	6.8(5.8 to 8.3)*	51.0(45.0 to 57.0)*
Yes (47)	7.5(6.1 to 9.3)	8.7(0.5 to 16.9)	71.9(45.4 to 114.1)*	67.4(53.7 to 81.1)*	9.6(5.4 to 8.1)*	67.4(53.7 to 81.1)

Data are expressed in percentages or value (95% CI).
Anti-TNF agents adalimumab, infliximab or etanercept; medications prescribed to less than 10% of the patients were not included. GMT significantly increased in controls, RA patients and all the subsets.
*p <0.05, subsets compared with control group;
†p<0.05, comparison with or without the specific medication within a group.
Factor increase in GMT after vaccination.
GMT, geometric mean titre; RA, rheumatoid arthritis; RF, rheumatoid factor; TNF, tumour necrosis factor.

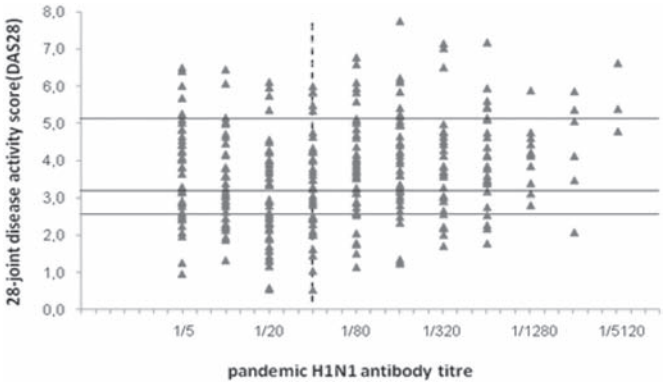

Figure 1 Disease activity and antibody response after vaccination against pandemic influenza A H1N1 (pH1N1) 2009. The vertical line indicates the 1/40 titer, inferior limit associated with seroprotection, and the continuos horizontal lines show the DAS 28 values corresponding to high disease activity (above 5.1), moderate disease activity (>3.2 and ≤5.1), low disease activity (>2.6 and ≤ 3.2) and remission (DAS28 < 2.6).

patients were not medicated and 32 (9.4%) were on monotherapy. Patients on methotrexate had the lowest responses (table 2).

After multivariate analyses, only age, RA and methotrexate were associated with impaired seroconversion (p<0.05). The probability of methotrexate-related reduction in seroconversion was 49% (OR 0.51; CI 95% 0.32 to 0.82; p=0.005).

Adverse events

The vaccine was well tolerated; no severe side effects were reported during follow-up. No patient sought extra consultations, although reporting significantly more adverse events (140 events/100 patients vs 87/100 controls, p<0.005). Minor local reactions were more frequent in controls (p=0.032) and mild systemic reactions (p=0.01) in patients (see supplementary table S4, available online only). Local symptoms prevailed during the first days (88%), while systemic symptoms clustered around the first 15 days (73%) postvaccination.

DISCUSSION

To the best of our knowledge, this is the largest study addressing adjuvant-free pH1N1 (2009) vaccine in active RA patients undergoing immunosuppressive treatment under routine care. Although our patients were predominantly women (seven women and one man), as usual in Brazilian RA series,[11] gender involvement is unlikely, because women reportedly have higher antibody titres to viral and bacterial vaccines.[12]

Age is a recognised factor in vaccine response. However, immunogenicity was similarly impaired in different age subsets and after age-matched subanalysis, as also reported for heterogeneous autoimmune rheumatic diseases.[7 13] Furthermore, after multivariate analysis, RA was an independent contributor to immune response impairment.

This reduced response could be related to the intrinsic RA immune state, disease activity, medication or lower vaccine immunogenicity. Disease activity neither precluded immune response nor was related to adverse events, a fact relevant to future vaccination guidelines.[1] As most patients were on combination therapy, neither RA itself nor medications could be independently assessed. Nevertheless, average/high effective doses of methotrexate were associated with reduced response at all endpoints. A recent study of adjuvanted pH1N1 vaccine also linked methotrexate to a lower antibody vaccine response,[14] while studies with seasonal trivalent vaccine showed no similar interference.[1 2 14–16]

We noted no effect of corticosteroids, traditional DMARD and TNF-blockers on vaccine immune response. Conversely, leflunomide and other immunosuppressors were associated with lower postvaccination GMT in an adjuvanted pandemic H1N1 vaccine trial.[14] In line with our observations, no interference of TNF-blockers was verified. Former trials with trivalent seasonal influenza vaccine reported no consistent influence of classic DMARD or corticosteroids.[1 2 14 16 17] Overall, TNF inhibitors lead to adequate or slightly reduced seroprotection rates.[1–3 14–19]

This study helped clarify the controversy of adjuvanted versus adjuvant-free vaccines, because our results are similar to those of one-dose adjuvanted pH1N1 vaccine in 82 RA patients.[13] In addition, when different preparations of influenza A H1N1 (2009) vaccine were tested in a large Chinese population, the adjuvant-free formulation was more effective with equivalent antigen content.[20]

Our data clearly settle the short-term safety of influenza A (pH1N1) 2009 vaccine in RA patients. Inflammatory tests remained unchanged and worsening of the disease, reported by nine out of 340 patients, cannot be distinguished from natural disease flares. The slight decrease in DAS28 and swollen joints after vaccination was well below the clinically significant value.[9] Moreover, our patients, under regular treatment aimed at disease control, were not preselected, regarding medications or duration of treatment, as our study was developed during the national anti-pH1N1 vaccination campaign.[5]

The observed lower seroconversion rate in RA patients, particularly on methotrexate, has broader implications and may place significant numbers of immunosuppressed individuals at risk of infection despite vaccination. Inferring from the improved response to a second dose (booster) of adjuvanted pH1N1 vaccine[13] and the response to seasonal influenza vaccine in RA patients, this study highlights the need for specific immunisation strategies for each new vaccine in this population.

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Competing interests None.

Ethics approval This study was conducted with the approval of the local Institutional Review Board, Faculdade Medicina, Universidade de São Paulo.

Provenance and peer review Not commissioned; externally peer reviewed.

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Table S1 – Inclusion and Exclusion criteria

Inclusion criteria	Exclusion criteria
≥ 18 years old	-previous confirmed infection with influenza A (pH1N1) 2009
-written informed consent	-anaphylactic response to vaccine components or to egg proteins,
-no changes in medication during the study	-acute febrile infection (temperature over 38ºC) at the time of vaccination
	-history of Guillain-Barré syndrome
	-history of demyelinating syndromes
	-previous vaccination with any live vaccine (four weeks before the study)
	-previous vaccination with any inactivated vaccine (two weeks before the study)
	-2010 seasonal influenza vaccination
	-hospitalized patients

Obs – confirmed infection with influenza A (pH1N1) 2009 = positive real time chain reaction of polymerase (rRT-PCR - endorsed by the World Health Organization (WHO), performed in combined nasopharyngeal and throat swabs or, in case of intubation, in the nasotracheal aspirate collection.

Table S2 Demographic characteristics, disease activity parameters and therapy before and after vaccination

	Total patients		**Stable disease**		**Patients reported worsening**	
	Before vaccination	**After vaccination**	**Before vaccination**	**After vaccination**	**Before vaccination**	**After vaccination**
n(%)	340(100)		331(97.4)		9(2.6)	
female n(%)	295(86.8)		287(86.7)		8(88.9)	
RF positive n(%)	249(72.8)		242(73.1)		7(77.7)	
Age (yrs)	55.8(11.5)		55.8(11.4)		56.6(15)	
Disease duration (yrs)	16.7(10.4)		16.7(10.5)		16.9(8.9)	
DAS28 -ESR	3.66 (1.35)	3.49 (1.36)†	3.67(1,37)	3.51(1.39)#	3.71(1.23)	5.11(1.57)*
Number tender joints	3.7(4.8)	3.7 (5.4)	3.9(5.2)	3.8(5.7)	3.8 (3.9)	9.8(10.1)*
Number swollen joints	4.4(4.60)	3.0 (3.87)†	4.4(4.7)	3.1(4.0)#	4.3 (3.6)	7.9(5.1)*
ESR (mm/h)	20.7(20.2)	20.6 (20.2)	20.5(20.2)	20.5(20.1)	18.3(10.9)	30.2(17.1)*
PGHA (0-100mm)	37.9(26.6)	36.9 (27.4)	38.5(26.7)	37.1(27.3)	30.2(24.1)	55.4(28.8)*
Pain (VAS -0-100mm)	38.3(27.0)	37.5 (28.2)	38,3(27.0)	37.4(28.2)	40.6(31.1)	51.0(28.3)*
CS n(%)	247(72.6)		240(72.3)		7 (77.8)	
Dose CS mg/day	8.6 (5.2)		8.5 (5.2)		7.9 (3.9)	
MTX n(%)	215(63.2)		211(63.7)		4(44.4)	
MTX mg/week	19.2 (5.6)		19.2 (5.6)		19.4 (4.3)	
LEF (20mg/day) n(%)	146(42.9)		141 (42.6)		5 (55.5)	
CHLOR n(%)	124 (36.5)		120(36.3)		4(44.4)	
Anti-TNF agents n(%)	47(13.8)		46(13.9)		1(11.1)	

Data are expressed as the mean (▫standard deviation) unless otherwise specified.
No significant differences between demographic characteristics and therapy between the three groups was observed. * # † before vs. after vaccination values; paired T test;$p<0.05$; RF, Rheumatoid Factor; DAS28, 28-joint disease activity score; ESR, erythrocyte sedimentation rate; PGHA, patient global health assessment; IGHA, investigator global health assessment; CS, corticosteroids; MTX, methotrexate; LEF, leflunomide; CHLOR, chloroquine; anti-TNF agents = adalimumab, infliximab, etanercept; medication prescribed to less than 10% of the patients were not included.

Table S3 Seroprotection and Seroconversion Rates and Factor Increase in the Geometric Mean Titres (GMT) after Influenza A pandemic (pH1N1) 2009 Vaccine in Rheumatoid Arthritis Patients and Controls (total population and age subsets)

total population			
Immunogenicity Endpoint	RA (n=340)	Control (n=234)	p-value
Factor Increase in GMT value (CI95%)	7.2 (6.1, 8.5)	13.2 (11.1, 15.8)	<.0001
Seroprotection rate % (CI95%)	60.0 (54.5, 65.3)	82.9 (77.5, 87.5)	<.0001
Seroconversion rate % (CI95%)	53.2 (47.8, 58.6)	76.9 (71.0, 82.2)	<.0001
Subset age less than or equal to 39yrs			
Immunogenicity Endpoint	RA (n=34)	Control (n=120)	p-value
Factor Increase in GMT value (CI95%)	7.4 (4.4, 12.4)	15.6 (12.1,12.9)	0.0081
Seroprotection rate % (CI95%)	61.8 (43.6, 77.8)	87.5 (80.2, 92.8)	0.0017
Seroconversion rate % (CI95%)	52.9 (35.2, 70.3)	80.8 (72.6, 87.4)	0.0017
subset age greater than 39yrs			
Immunogenicity Endpoint	RA (n=306)	Control (114)	p-value
Factor Increase in GMT value (CI95%)	7.3 (6.0, 8.6)	11.1 (8.8, 14.04)	0.0039
Seroprotection rate % (CI95%)	59.8 (54.1, 65.3)	78.1 (69.4, 85.3)	<.0001
Seroconversion rate % (CI95%)	53.4 (47.5, 59.0)	72.8 (63.7, 80.7)	<.0001

Data are expressed in % or value (95% confidence interval). Age subsets determined according with the median age in the control group. GMT - geometric mean titre

Table S4 - Adverse Events of Influenza A pandemic (pH1N1) 2009 Vaccination in Rheumatoid Arthritis (RA) and Control Patients

	Control n=234 (%)	**RA n=336 (%)**	***p-Value***
local symptoms	35 (15.0)	30 (8.9)	0.032
fever/tremors	10 (4.3)	39 (11.6)	0.0021
arthralgia/myalgia	25 (10.7)	92 (27.4)	0.0001
Flu-like	30 (12.8)	73 (21.7)	0.0077

Values expressed as number of patients and %. Four RA patients had incomplete diary-cards and were excluded from this analysis. Local symptoms = presence of one or more of the following: pain, redness, swelling, and itching; Flu-like= presence of one or more of the following: sore throat, cough, rhinorrhea, and nasal congestion.

Anexos D – Outras publicações relacionadas à tese

1. Ribeiro AC, Laurindo IM, Guedes LK, Saad CG, Moraes JC, Silva CA et al. Abatacept and reduced immune response to pandemic 2009 influenza A/H1N1 vaccination in patients with rheumatoid arthritis. Arthritis Care Res 2013;65:476-80.
2. Saad CG, Borba EF, Aikawa NE, Silva CA, Pereira RM, Calich AL et al. Immunogenicity and safety of the 2009 non-adjuvanted influenza A/H1N1 vaccine in a large cohort of autoimmune rheumatic diseases. Ann Rheum Dis 2011;70:1068-73.
3. Borba EF, Saad CG, Pasoto SG, Calich AL, Aikawa NE, Ribeiro AC et al. Influenza A/H1N1 vaccination of patients with SLE: can antimalarial drugs restore diminished response under immunosuppressive therapy? Rheumatology 2012;51:1061-9.
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5. Shinjo SK, de Moraes JC, Levy-Neto M, Aikawa NE, de Medeiros Ribeiro AC, Schahin Saad CG et al. Pandemic unadjuvanted influenza A (H1N1) vaccine in dermatomyositis and polymyositis: immunogenicity independent of therapy and no harmful effect in disease. Vaccine 2012;31:202-6.

6. Pasoto SG, Ribeiro AC, Viana VS, Leon EP, Bueno C, Neto ML et al. Short and long-term effects of pandemic unadjuvanted influenza A(H1N1)pdm09 vaccine on clinical manifestations and autoantibody profile in primary Sjögren's syndrome. Vaccine 2013;31:1793-8.

7. Miossi R, Fuller R, Moraes JC, Ribeiro AC, Saad CG, Aikawa NE, et al. Immunogenicity of influenza H1N1 vaccination in mixed connective tissue disease: effect of disease and therapy. Clinics 2013;68:129-34.

8. Aikawa NE, Campos LM, Goldenstein-Schainberg C, Saad CG, Ribeiro AC, Bueno C et al. Effective seroconversion and safety following the pandemic influenza vaccination (anti-H1N1) in patients with juvenile idiopathic arthritis. Scand J Rheumatol 2013;42:34-40.

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BRIEF REPORT

Abatacept and Reduced Immune Response to Pandemic 2009 Influenza A/H1N1 Vaccination in Patients With Rheumatoid Arthritis

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Objective. **To evaluate the influence of abatacept (ABA) and associated contributing factors on pandemic 2009 influenza A/H1N1 vaccine immunogenicity in rheumatoid arthritis (RA) patients.**

Methods. **The response to a nonadjuvanted monovalent pandemic 2009 influenza A/H1N1 killed virus vaccine was analyzed in 11 RA patients using ABA (RA-ABA), most with concomitant nonbiologic disease-modifying antirheumatic drugs (DMARDS), and compared to 33 age-matched RA patients on methotrexate (MTX) and 55 healthy controls, all without previous seroprotection. Clinical and laboratory evaluations were performed before and 21 days after vaccination. Anti-influenza antibody titers were measured by hemagglutination inhibition assay. Seroprotection (antibody titers ≥1:40) and the factor increase (FI) in the geometric mean titers (GMTs) were calculated. Prevaccination lymphocyte counts and gammaglobulin levels were determined.**

Results. **Sex distribution, disease duration, and the Disease Activity Score in 28 joints were similar in the RA groups ($P > 0.05$). After vaccination, seroprotection was significantly reduced in RA-ABA patients compared to RA-MTX patients (9% versus 58%; $P = 0.006$) and controls (69%; $P \leq 0.001$). FI-GMT was severely reduced in RA-ABA patients compared to RA-MTX patients (1.8 [1.4–2.3] versus 8.7 [5.2–17.4]; $P < 0.001$) and controls (11.5 [8.0–16.7]; $P \leq 0.001$). Lymphocyte counts were comparable in RA groups ($P > 0.05$), but RA-ABA patients had slightly lower gammaglobulin levels than RA-MTX patients (0.9 gm/dl [0.6–1.8] versus 1.2 gm/dl [0.8–1.7]; $P = 0.03$), although almost all were within the normal range values.**

Conclusion. **The current study established that ABA, in association with traditional DMARDs, significantly reduces the humoral response to pandemic 2009 influenza A/H1N1 vaccine in RA patients. The results suggest an influence of costimulatory modulation in humoral response to this vaccine.**

Introduction

Rheumatoid arthritis (RA) treatment can induce immunosuppression and increase the risk of infection (1). Abatacept (ABA) is a soluble fusion protein that selectively modulates the CD80/CD86:CD28 costimulatory signal required for full T cell activation (2).

Recently, our group studied the response to a nonadjuvanted antipandemic 2009 influenza A/H1N1 vaccine in a large Brazilian cohort of 1,668 autoimmune rheumatic disease patients. The results demonstrated a diminished immunogenicity to this vaccine in RA patients, especially those undergoing methotrexate (MTX) therapy (3,4). Other trials involving the use of adjuvanted pandemic influenza vaccines also showed that the use of disease-modifying antirheumatic drugs (DMARDs) (5), including MTX (5,6) and leflunomide (5,7), could impair the response. In regard to biologic therapies, tumor necrosis factor inhibitors were reported to have a limited impact on the pandemic influenza vaccination (4–7), while rituximab was associated

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Significance & Innovations

- The efficacy of pandemic influenza A/H1N1 vaccines has not been properly evaluated in rheumatoid arthritis (RA) patients under treatment with abatacept.
- The immunogenicity of a nonadjuvanted pandemic 2009 influenza A/H1N1 vaccine in RA patients treated with such biologic therapy was studied and compared to age-matched RA patients treated with methotrexate and to healthy controls.
- Prevaccination lymphocyte counts and gammaglobulin levels were also evaluated.

with a significant reduction on the humoral response (5,6). There are, however, limited data about the impact of ABA in the influenza vaccine with only 1 report using adjuvanted vaccine, suggesting a negative effect (6). The study included a heterogeneous group of patients, which may have hampered the interpretation of findings because diverse immunogenicity related to rheumatic disease was observed in patients immunized with the pandemic vaccine (3,6,7). Moreover, the inclusion of a specific age-matched healthy control group and an RA group under treatment only with traditional DMARDs is essential to minimize the influence of age (8) and discriminate the effect of each therapy (4–7).

Thus, the aim of this report is to assess the influence of ABA and associated contributing factors on the immune response to a nonadjuvanted pandemic 2009 influenza A/H1N1 vaccine. The results were compared to those of age-matched healthy controls and RA patients using only traditional DMARDS.

Patients and methods

Study design and participants. This is a subanalysis of a prospective study conducted at a single center in São Paulo, Brazil, during the Public National Health pandemic 2009 influenza A/H1N1 vaccination campaign, as described elsewhere (3). It was approved by the Institutional Review Board. In the prior study, 340 RA patients (according to the 1987 American College of Rheumatology criteria) (9), ages ≥18 years and seen for regular followup at the Rheumatology Outpatient Clinic of the Hospital das Clínicas da Universidade de São Paulo, were included and compared to healthy controls. In this subanalysis, 11 RA patients treated with ABA (RA-ABA) were compared to age-matched control groups, i.e., RA patients treated with MTX (RA-MTX) and healthy controls. The exclusion criteria were described elsewhere (3,4).

The vaccine, Sanofi Pasteur Influenza A/H1N1, was a nonadjuvanted monovalent pandemic 2009 influenza A/H1N1 killed virus vaccine (A/California/7/2009/Butantan Institute/Sanofi Pasteur, São Paulo, Brazil) containing 15μg hemagglutinin from an influenza A/California/07/2009(H1N1) virus-like strain (NYMCx-179A) per 0.5-ml dose (3,4).

Study procedures. All subjects received a single intramuscular dose of the vaccine. Blood samples were collected before and 21 days after the vaccination; the erythrocyte sedimentation rate (ESR), C-reactive protein level, and antibody analyses, as well as the clinical evaluation of disease activity (Disease Activity Score in 28 joints using the ESR) were performed. Prevaccination collection of whole blood for lymphocyte count (automated standard cell blood count and differential) and for analysis of total immunoglobulin levels (semiquantitative electrophoretic separation by densitometry) was also performed. Pre-ABA lymphocyte counts and total immunoglobulin levels, measured by the same techniques, were available by routine screening for patients beginning/changing any biologic treatment and were retrospectively assessed. Regarding safety assessments, a 21-day symptom diary card for prospective completion was given to each participant following vaccination and returned 21 days after vaccination (3,4).

Hemagglutination inhibition assay. The influenza antigen used was the H1N1 A/California/7/2009 supplied by the Butantan Institute. Antibody titers were obtained at baseline and 21 days after immunization in all groups. Two serologic end points were calculated: the postvaccination seroprotection rate (percentage of patients with titers ≥1:40) and the seroconversion rate (percentage of patients with a ≥4-fold increase in vaccination titers if the prevaccination titers were ≥1:10 or titers ≥1:40 if the prevaccination titers were <1:10). The antibody geometric mean titers (GMTs) and the factor increase (FI) in the GMTs were also calculated.

Statistical analysis. Age-matching of the RA-MTX group and healthy controls was carried out by random selection using SPSS software, version 15. GMTs and FI-GMT were calculated and analyzed using log-transformed data. Categorical variables were compared by Fisher's exact test or chi-square test as appropriate. Normally or non-normally distributed variables were compared using the *t*-test and Wilcoxon's rank sum test, respectively. When comparisons of continuous variables were performed among more than 2 groups, one-way analysis of variance or Kruskal-Wallis analysis of variance was used as appropriate. All tests were 2-sided (alpha level of 0.05).

Results

Baseline data. The 11 RA-ABA patients were age-matched to random RA-MTX patients (n = 33) and healthy controls (n = 55), and all of them completed the study. To overcome previous seroprotection as a confounder, none of the subjects included had discernible levels of seroprotective antibodies before vaccination. RA-ABA patients were similar to RA-MTX patients regarding age, sex, disease duration, disease activity, inflammatory markers, concomitant use of leflunomide or chloroquine, and total number of DMARDs taken. There was, however, a lower frequency of MTX use in patients under treatment with ABA ($P < 0.05$). The healthy control group had comparable age and female sex predominance (Table 1).

Table 1. Demographic characteristics and disease activity parameters before pandemic 2009 influenza A/H1N1 vaccination and therapy of RA patients at baseline*

	RA-ABA (n = 11)	RA-MTX (n = 33)	Controls (n = 55)	*P*
Female	11 (100)	29 (88)	42 (76)	0.11†
Age, years	55 (27–75)	56 (29–74)	52 (27–80)	0.49†
RA parameters				
RF positivity	5 (45)	25 (76)	–	0.13
Anti-CCP positivity	6 (55)	28 (85)	–	0.26
Disease duration, years	17 (8–28)	12 (1–34)	–	0.19
DAS28-ESR	3.8 (2.5–6.0)	3.6 (1.2–7.2)	–	0.98
ESR, mm/hour	34 (7–78)	40 (0–100)	–	0.06
CRP level, mg/dl	5.9 (1.5–41.7)	7.8 (1.0–53.4)	–	0.67
Treatment				
GC	9 (82)	28 (85)	–	1
GC dose, mg/day	7.5 (5–10)	10 (2.5–40)	–	0.89
MTX	6 (55)	33 (100)	–	< 0.001
MTX dose, mg/week	25 (20–25)	25 (15–25)	–	0.43
LEF	4 (36)	6 (18)	–	0.24
CHLOR	3 (27)	15 (46)	–	0.48
DMARDs	2 (1–3)	2 (1–3)	–	0.11

* Values are the absolute number (frequency) or the median (range). RA = rheumatoid arthritis; ABA = abatacept; MTX = methotrexate; RF = rheumatoid factor; anti-CCP = anti–cyclic citrullinated peptide; DAS28 = Disease Activity Score in 28 joints; ESR = erythrocyte sedimentation rate; CRP = C-reactive protein; GC = glucocorticoids; LEF = leflunomide; CHLOR = chloroquine; DMARDs = disease-modifying antirheumatic drugs.
† Related to concomitant comparison of the 3 groups.

ABA. RA-ABA patients received the recommended doses according to weight (2). The median (range) duration of treatment was 34 weeks (4–273 weeks). Eight (73%) patients had been taking ABA for more than 24 weeks. The median (range) time since the last dose was 18 days (0–21 days) prior to the vaccination. Nevertheless, as ABA is administered every 4 weeks, each patient received 1 dose during the 3-week period of the study.

Response to immunization. Prevaccination GMTs were very low and similar in all groups (Table 2). Seroconversion was not obtained in any of the RA-ABA patients, and only 1 subject (9%) achieved seroprotection. These trends were significantly different from those observed in other groups ($P < 0.001$ for seroconversion and $P = 0.001$ for seroprotection). Despite a significant and slight increase in GMT (6.0 [95% confidence interval (95% CI) 4.6–7.9] to 10.7 [95% CI 7.2–15.7]; $P = 0.008$) after vaccination, FI-GMT ($P < 0.001$) and postvaccination GMT ($P < 0.001$) were severely reduced in the RA-ABA group compared to the other groups (Table 2). RA-MTX patients and controls had more significant increases in GMT after vaccination (6.0 [95% CI 5.3–6.9] to 52.6 [95% CI 31.5–87.7]; $P < 0.001$ and 6.6 [95% CI 5.8–7.5] to 76.1 [95% CI 52.9–109.3]; $P < 0.001$, respectively). In all parameters analyzed, RA-MTX patients exhibited lower responses than controls, but these differences did not reach statistical significance. No correlation was observed between any of the end points and the duration of treatment with ABA or time since the last dose ($P > 0.05$).

Lymphocyte counts and gammaglobulin levels. Prevaccination lymphocyte counts were similar in both RA groups ($P = 0.73$) (Table 3). In contrast, RA-ABA patients

Table 2. Serologic data before and after pandemic 2009 influenza A/H1N1 vaccination of RA patients and controls*

	RA-ABA (n = 11)	RA-MTX (n = 33)	Controls (n = 55)	*P*†
Before vaccination				
GMT (95% CI)	6.0 (4.6–7.9)	6.0 (5.3–6.9)	6.6 (5.8–7.5)	0.77
Seroprotection	0	0	0	1.0
After vaccination				
Seroconversion	0‡	19 (58)	36 (66)	< 0.001
Seroprotection	1 (9)§	19 (58)	38 (69)	0.001
GMT (95% CI)	10.7 (7.2–15.7)‡	52.6 (31.5–87.7)	76.1 (52.9–109.3)	< 0.001
FI-GMT (95% CI)	1.8 (1.4–2.3)‡	8.7 (5.2–17.4)	11.5 (8.0–16.7)	< 0.001

* Values are the absolute number (frequency) unless otherwise indicated. RA = rheumatoid arthritis; ABA = abatacept; MTX = methotrexate; GMT = geometric mean titer; 95% CI = 95% confidence interval; FI = factor increase in GMT after vaccination. GMT significantly increased in all RA subsets and in controls ($P < 0.05$).
† Related to the concomitant comparison of all 3 groups.
‡ $P < 0.001$ (RA-ABA vs. RA-MTX); $P \leq 0.001$ (RA-ABA vs. controls).
§ $P = 0.006$ (RA-ABA vs. RA-MTX); $P \leq 0.001$ (RA-ABA vs. controls).

Table 3. Lymphocyte counts and gammaglobulin levels before vaccination in the RA groups and pretreatment gammaglobulin level in the RA-ABA group*

	RA-ABA (n = 11)	RA-MTX (n = 33)
Prevaccination lymphocyte count (cells/mm^3) (normal range 900–3,400)	2,000 (1,000–4,000)	2,100 (800–5,000)
Prevaccination gammaglobulin level (gm/dl) (normal range 0.7–1.5)	0.9 (0.6–1.8)†	1.2 (0.8–1.7)
Pre-ABA gammaglobulin level (gm/dl) (normal range 0.7–1.5)	1.3 (0.9–2.3)	–

* Values are the median (range). RA = rheumatoid arthritis; ABA = abatacept; MTX = methotrexate.
† $P = 0.03$, RA-ABA vs. RA-MTX; $P = 0.004$, prevaccination vs. pre-ABA levels within the RA-ABA group.

had prevaccination gammaglobulin levels lower than those observed in RA-MTX patients ($P = 0.03$) (Table 3), but only 1 patient presented with a value (0.6 gm/dl) below normal range (0.7–1.5) and 4 patients presented with a borderline level (0.7 gm/dl), a value at the limit of normal range. The RA-MTX group had no patient with low or borderline gammaglobulin levels. Of note, the 8 patients under treatment with ABA for more than 24 weeks had significantly lower median gammaglobulin levels compared to those treated for a shorter length of time (0.7 gm/dl [range 0.6–1.2] versus 1.1 gm/dl [range 1.1–1.8]; $P = 0.048$). In addition, gammaglobulin levels measured and available in all RA-ABA patients immediately before ABA treatment were within the normal range and statistically higher ($P = 0.004$) than prevaccination gammaglobulin levels, with no patient showing low or borderline levels immediately before ABA treatment (Table 3).

Regarding adverse events, severe side effects were not reported during the followup period. The rates of minor side effects were comparable: 55% in RA-ABA patients, 39% in RA-MTX patients, and 40% in control groups ($P = 0.64$).

Discussion

The current study established that ABA significantly reduces the humoral response to pandemic 2009 influenza A/H1N1 vaccine in RA patients. The slightly lower immunoglobulin levels observed in these patients could partially explain this alteration.

The remarkable impact of costimulatory modulation on the humoral response was evidenced by all the parameters analyzed herein in RA-ABA patients: the uniform lack of seroconversion, the striking reduction in seroprotection, and the low GMTs and FI-GMT. These findings reinforce a single previous published study using adjuvanted pandemic 2009 influenza A/H1N1 vaccine, which also demonstrated a reduced humoral response in a heterogeneous group of inflammatory arthritis patients under ABA treatment (6). In fact, studies with animal models revealed a critical role for CD80/86:CD28 costimulation during the immune response to different antigens (10,11), and the response to the T cell–dependent neoantigens bacteriophage X174 and keyhole limpet hemocyanin (KLH) was reduced in psoriasis patients treated with ABA, without inducing tolerance (12).

In contrast, few trials failed to show a major reduction in the immune response to other antigens. Healthy individuals who received a single dose of ABA had a decreased, but not significantly inhibited, response to tetanus toxoid and 23-valent pneumococcal vaccines (13), probably explained by the very limited ABA exposure, without combination therapy. Furthermore, studies with pneumococcal (14) and seasonal influenza (15) vaccines with a limited number of RA patients were performed, without control groups, suggesting an adequate response. Regarding pneumococcal vaccine, the polysaccharide and less T cell–dependent nature of the antigen may account for the preserved immune response during costimulatory modulation with ABA. Regarding seasonal influenza vaccine, previous exposure to such viral or vaccine antigens may explain the results, whereas none of the subjects evaluated herein were seroprotected before immunization, which is what makes this pandemic influenza vaccine comparable to a pure neoantigen. It remains to be determined if a second boost would improve vaccine response in these patients as demonstrated previously in patients under DMARDs and other biologic therapies (5).

This result is strengthened by the inclusion of the relevant control groups since RA patients frequently use ABA in combination with traditional DMARDs. Because MTX was associated with a reduced response to the pandemic influenza vaccine (4–6), it was therefore crucial to include an RA age-matched group under treatment with MTX for comparison. Additionally, an age-matched healthy control group was necessary to avoid the known influence of this parameter on pandemic influenza vaccine immunogenicity (8).

Lymphopenia was not detected in these patients and cannot account for the compromised vaccine immunogenicity, whereas the observed slightly lower gammaglobulin levels in RA-ABA patients may have influenced pandemic influenza vaccine response or reflected the same subjacent mechanism. Responses to the pandemic influenza vaccine and to the neoantigen KLH were also reported to be decreased in RA patients treated with rituximab, a well-established inhibitor of the humoral immune system (5,6,16).

An indication bias favoring a correlation between ABA use and reduced gammaglobulin levels in patients referred for ABA therapy does not seem to be a likely explanation for the lower gammaglobulin levels in RA-ABA patients, given that the pre-ABA gammaglobulin levels were within

the normal range, with no patient showing low or borderline levels. On the other hand, the significant prevaccination lower levels of gammaglobulin, although within normal range for all but 1 patient, seemed related to long-term ABA use. These novel findings need to be confirmed as a drug effect. In addition, we cannot exclude the drug synergism between ABA and traditional DMARDs, since the majority of patients were under combination therapy.

The small number of patients represents a limitation of the findings. Convenience samples are prone to selection bias and low extern validation. On the other hand, such a severe reduction in humoral responses in a small sample may imply a robust drug effect.

In conclusion, inhibition of costimulation mediated through CD80/86:CD28 in association with traditional DMARDs was related to a severe reduction in the humoral response to influenza A/H1N1 vaccine in RA patients.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be submitted for publication. Dr. Ribeiro had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Ribeiro, Laurindo, Guedes, Saad, Moraes, Silva, Bonfa.

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Analysis and interpretation of data. Ribeiro, Laurindo, Bonfa.

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Immunogenicity and safety of the 2009 non-adjuvanted influenza A/H1N1 vaccine in a large cohort of autoimmune rheumatic diseases

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ABSTRACT

Background Despite the WHO recommendation that the 2010–2011 trivalent seasonal flu vaccine must contain A/California/7/2009/H1N1-like virus there is no consistent data regarding its immunogenicity and safety in a large autoimmune rheumatic disease (ARD) population.

Methods 1668 ARD patients (systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), ankylosing spondylitis (AS), systemic sclerosis, psoriatic arthritis (PsA), Behçet's disease (BD), mixed connective tissue disease, primary antiphospholipid syndrome (PAPS), dermatomyositis (DM), primary Sjögren's syndrome, Takayasu's arteritis, polymyositis and Granulomatosis with polyangiitis (Wegener's) (GPA)) and 234 healthy controls were vaccinated with a non-adjuvanted influenza A/California/7/2009(H1N1) virus-like strain flu. Subjects were evaluated before vaccination and 21 days post-vaccination. The percentage of seroprotection, seroconversion and the factor increase in geometric mean titre (GMT) were calculated.

Results After immunisation, seroprotection rates (68.5% vs 82.9% $p<0.0001$), seroconversion rates (63.4% vs 76.9%, $p<0.001$) and the factor increase in GMT (8.9 vs 13.2 $p<0.0001$) were significantly lower in ARD than controls. Analysis of specific diseases revealed that seroprotection significantly reduced in SLE ($p<0.0001$), RA ($p<0.0001$), PsA ($p=0.0006$), AS ($p=0.04$), BD ($p=0.04$) and DM ($p=0.04$) patients than controls. The seroconversion rates in SLE ($p<0.0001$), RA ($p<0.0001$) and PsA ($p=0.0006$) patients and the increase in GMTs in SLE ($p<0.0001$), RA ($p<0.0001$) and PsA ($p<0.0001$) patients were also reduced compared with controls. Moderate and severe side effects were not reported.

Conclusions The novel recognition of a diverse vaccine immunogenicity profile in distinct ARDs supports the notion that a booster dose may be recommended for diseases with suboptimal immune responses. This large study also settles the issue of vaccine safety. (ClinicalTrials.gov #NCT01151644)

INTRODUCTION

Since the identification of a novel influenza A H1N1 virus in Mexico and the USA in April 2009, the virus has spread throughout the world, prompting the WHO to raise the pandemic alert level to phase VI on 11 June 2009.[1] This flu pandemic caused by the new A/H1N1 virus led to a higher incidence of hospitalisation and death than the annual rates associated with seasonal flu viruses.[1] Recently, the WHO recommended that the Northern Hemisphere's 2010–2011 and Southern Hemisphere's 2011 trivalent seasonal flu vaccine must contain A/California/7/2009(H1N1)-like virus.[2]

According to the 2010 Recommendations of the Advisory Committee on Immunization Practices and the recent European League Against Rheumatism recommendation,[3] immunocompromised patients are recommended to receive the flu vaccine (A/California/7/2009), as these patients are likely to have a higher risk and a more severe course of H1N1 infection.[4] Despite this recommendation, the immunogenicity of this vaccine in this patient population remains unclear.

Previous studies that evaluated non-rheumatic immunosuppressed patients lacked a normal control population for comparison. Nevertheless, the immune response to the pandemic H1N1 flu vaccine seems to be low for HIV-infected patients[5 6] and recipients of stem cell transplants.[7] However, pre-vaccination data were not available for the latter group, and safety assessments were not uniformly reported for either of these populations, thus preventing a definitive conclusion to be drawn from these findings.[6 7]

With regard to autoimmune rheumatic diseases (ARDs), there is only one published study that evaluated the efficacy and safety of the vaccine against pandemic influenza A H1N1/2009 vaccine in a very limited number of systemic lupus patients.[8] The dearth of data regarding other rheumatic diseases and, more importantly, the need for an appropriate patient sample size led to the development of this research.

The objectives of this study are to evaluate the humoral response and safety of the pandemic influenza A H1N1/2009 vaccine in immunosuppressed patients with ARDs compared with healthy controls.

METHODS

Study design

This is a prospective study conducted at a single site in São Paulo, Brazil (Outpatient Clinics of

Rheumatology Division, Hospital das Clínicas da Universidade de São Paulo) between March 2010 and April 2010. The protocol was approved by the Local Institutional Review Board and registered with Clinicaltrials.gov under #NCT01151644.

All ARD patients who were regularly followed at the rheumatology outpatient clinics were invited by letter to participate in the Public Health influenza A H1N1/2009 vaccine campaign at the Immunization Center of the Hospital das Clínicas da Universidade de São Paulo. Healthy individuals who came to this centre seeking vaccination in response to the Public Health National Campaign were invited to participate in the control group.

The study included two phases: entry (vaccination from 22 March to 2 April) and a follow-up period of 21 days with a personal diary card of side effects. Blood samples were obtained from each participant immediately before and 21 days after vaccination.

Participants

A total of 1668 patients with ARD were included in this study. All patients fulfilled the international classification criteria: systemic lupus erythematosus (SLE),[9] rheumatoid arthritis (RA),[10] ankylosing spondylitis (AS),[11] systemic sclerosis (SSc),[12] psoriatic arthritis (PsA),[13] Behçet's disease (BD),[14] mixed connective tissue disease (MCTD),[15] primary antiphospholipid syndrome (PAPS),[16] dermatomyositis (DM),[17] primary Sjögren's syndrome (pSS),[18] Takayasu's arteritis (TA),[19] polymyositis (PM)[17] and Granulomatosis with polyangiitis (Wegener's) (GPA).[20] A total of 234 healthy subjects were concomitantly included in the control group.

Inclusion and exclusion criteria

All participants were ≥18 years old and provided written informed consent. Exclusion criteria were as follows: previous known infection with influenza A (H1N1) 2009; anaphylactic response to vaccine components or to egg; acute infection resulting in fever over 38°C at the time of vaccination; history of Guillain–Barré syndrome or demyelinating syndromes; previous vaccination with any live vaccine 4 weeks before or any inactivated vaccine 2 weeks before the study; 2010 seasonal flu vaccination; or blood transfusion within 6 months and hospitalised patients.

Vaccine

The H1N1 vaccine, a novel monovalent, unadjuvanted, inactivated, split-virus vaccine, was produced by Butantan Institute/Sanofi Pasteur (São Paulo, Brazil). The active substance is an inactivated split flu virus containing antigen equivalent to the A/California/7/2009(H1N1) virus-like strain (NYMCx-179A), one of the candidate reassortant vaccine viruses recommended by the WHO. The vaccine was prepared in embryonated chicken eggs using the same standard techniques for the production of seasonal, trivalent, inactivated vaccine and was presented in 5 ml multi-dose vials with thimerosal added as a preservative (45 µg per 0.5 ml dose).

Study procedures

All participants received a single intramuscular dose (0.5 ml) of 15 µg of haemagglutinin antigen specific for pandemic H1N1 A/California/7/2009-like virus (A/California/7/2009/Butantan Institute/Sanofi Pasteur).

Safety assessments

A 21-day diary card was given to each participant upon entry into the study which included 13 established side effects requiring yes/no responses. This written card included the following: local reactions (pain, redness, swelling and itching) and systemic adverse events, such as arthralgia, fever, headache, myalgia, sore throat, cough, diarrhoea, rhinorrhoea and nasal congestion. Participants were asked to return their diary cards at the end of the follow-up period (21 days after vaccination). All local reactions were considered to be related to the H1N1 vaccine. Recorded systemic symptoms were checked by the investigators to determine the causality of solicited systemic adverse events. Unsolicited adverse events were also assessed. Severe side effects were defined as those requiring hospitalisation or death.

Disease activity

Pre-vaccination disease activity was assessed in SLE patients by the SLE Disease Activity Index (SLEDAI)[21] and laboratory assessment of RA activity was done using erythrocyte sedimentation rate (ESR) and C reactive protein (CRP) levels.

Laboratory assays

Blood samples were collected at baseline and 3 weeks after vaccination. The immunogenicity of the H1N1 A/California/7/2009-like virus vaccine was evaluated using the haemagglutination inhibition assay (HIA) at the Adolfo Lutz Institute.

Haemagglutination inhibition assay

The flu virus antigen used in this study was the H1N1 A/California/7/2009 supplied by Butantan Institute. Virus concentrations were previously determined by haemagglutinin antigen titration, and the HIA test was performed after removing the naturally occurring non-specific inhibitors from the sera as previously described.[22] The immune response to H1N1 vaccination was evaluated by determining the levels of antibodies by HIA. Anti-H1N1 titre was determined by influenza HIA. Percentage of seroprotection (titre ≥1:40), seroconversion (pre-vaccination titre <1:10 and post-vaccination HIA titre ≥1:40 or pre-vaccination titres ≥1:10 and post-vaccination titres ≥4-fold increase), geometric mean titres (GMTs) and factor increase in GMTs were calculated.

Statistical analysis

The sample size was chosen practically rather than statistically because of the need to obtain robust estimates of vaccine immunogenicity and safety in ARD. The large sample size of ARD population and controls gave the study a power to find differences between frequencies inferior to 1% (with a power of performed test >80%).

A subanalysis of patients and age-matched controls was performed by a random selection of ARD patients using the SPSS (version 15).

The analyses were descriptive, with calculation of two-sided 95% CI, assuming binomial distributions for dichotomous variables and log-normal distribution for haemagglutination inhibition titres. For categorical variables, statistical summaries included the rate of seroconversion and seroprotection; these were compared using Fisher's exact test. Every subgroup of volunteers had the haemagglutination inhibition GMT calculated before and 21 days after vaccination. Compared between

each subgroup of patients with ARD and the group of healthy volunteers using a two-sided Student's t test with an α level of 0.05.

Multivariate logistic regression analyses were performed to analyse possible factors that influenced the vaccine performance.

RESULTS

A total of 1862 patients were recruited of whom 1668 (89.6%) completed the study. The different categories of ARD patients included those with SLE (n=572), RA (n=343), AS (n=152), SSc (n=127), PsA (n=101), BD (n=85), MCTD (n=69), PAPS (n=54), DM (n=45), pSS (n=36), TA (n=30), PM (n=28) and WG (n=26) who were compared with 234 healthy controls (table 1).

Table 1 Distribution of rheumatic diseases and therapy

	Patients (n=1668)
Disease	
Systemic lupus erythematosus	572 (34.3)
Rheumatoid arthritis	343 (20.6)
Ankylosing spondylitis	152 (9.1)
Systemic sclerosis	127 (7.6)
Psoriatic arthritis	101 (6.1)
Behçet's disease	85 (5.1)
Mixed connective tissue disease	69 (4.1)
Primary antiphospholipid syndrome	54 (3.2)
Dermatomyositis	45 (2.7)
Primary Sjögren's syndrome	36 (2.2)
Takayasu's arteritis	30 (1.8)
Polymyositis	28 (1.7)
Wegener's granulomatosis	26 (1.6)
Treatment	
Prednisone use	729 (43.7)
Prednisone dose (mg/day)	5.4±9.3
Prednisone ≥20 mg/day	153 (9.2)
Immunosuppressant use	942 (56.5)

Data are expressed as the mean±SD or n (%).

A significantly higher mean age (47.1±14.1 vs 38.7±12.5 years, p<0.0001) and a predominance of women (80.4% vs 65.8%, p<0.0001) were observed in ARD patients compared with controls.

Approximately half of the patients were under glucocorticoid therapy, receiving a mean daily dose of 5.4±9.3 mg prednisone/day, and less than 10% of these patients were under high doses (≥20 mg/day). More than 50% of all ARD patients were using immunosuppressive drugs (table 1).

The overall vaccine immune response is illustrated in table 2. Prior to immunisation, the seroprotection rate was comparable between ARD patients and controls (p=0.57). After immunisation, the seroprotection (68.5%, 95% CI 66.3% to 70.7% vs 82.9%, 95% CI 78.1% to 87.7%, p<0.0001) and seroconversion rates (63.4%, 95% CI 61.1% to 65.7% vs 76.9%, 95% CI 71.5% to 82.3%, p<0.001) were significantly lower in ARD patients compared with healthy subjects. Subanalysis of 234 randomly selected patients and 234 age-matched controls (mean age of 38.7±12.6 years) revealed that results still hold with comparable seroprotection prior to immunisation (p=0.28) and lower seroprotection after immunisation (p=0.001) as well as lower seroconversion rates (p=0.01) in ARD patients compared with controls.

Analysis of each disease subgroup revealed that pre-vaccination seroprotection was similar in patients and controls (p>0.05). After vaccination, seroprotection rates were significantly reduced in SLE (p<0.0001), RA (p<0.0001), PsA (p=0.0006), AS (p=0.04), BD (p=0.04) and DM (p=0.04) patients. Seroconversion rates were also reduced in SLE (p<0.0001), RA (p<0.0001) and PsA (p=0.0006) patients compared with healthy subjects.

GMTs before immunisation (8.0, 95% CI 7.7 to 8.4 vs 9.3, 95% CI 8.2 to 10.5, p=0.016), GMTs after immunisation (71.5, 95% CI 66.2 to 77.3 vs 122.9, 95% CI 103.4 to 146.1, p<0.0001) and the factor increase in GMTs (8.9, 95% CI 8.3 to

Table 2 Seroprotection and seroconversion rates of influenza A (H1N1) 2009 vaccine in ARD patients and controls

		Seroprotection rate (titre ≥1/40)		
		Before immunisation	After immunisation	
	N	% (95% CI)		Seroconversion rate
ARD	1668	10.3 (8.8 to 11.8)	68.5 (66.3 to 70.7)*	63.4 (61.1 to 65.7)*
SLE	572	8.9 (6.6 to 11.2)	64.9 (61.0 to 68.8)*	60.5 (56.5 to 64.5)*
RA	343	10.8 (7.5 to 14.1)	60.1 (54.9 to 65.3)*	53.4 (48.1 to 58.7)*
PsA	101	7.9 (2.6 to 13.2)	65.3 (56.0 to 74.6)*	57.4 (47.8 to 67.0)*
AS	152	8.6 (4.1 to 13.1)	73.7 (66.7 to 80.7)*	69.8 (62.5 to 77.1)
BD	85	7.1 (1.6 to 12.6)	71.8 (62.2 to 81.4)*	69.4 (59.6 to 79.2)
DM	45	8.9 (0.6 to 17.2)	68.9 (55.4 to 82.4)*	66.7 (52.9 to 80.5)
SSc	127	18.1 (11.4 to 24.8)	81.1 (74.3 to 87.9)	73.2 (65.5 to 80.9)
MCTD	69	10.1 (3.0 to 17.2)	75.4 (65.2 to 85.6)	68.1 (57.1 to 79.1)
PAPS	54	5.6 (−0.5 to 11.7)	79.6 (68.8 to 90.3)	77.8 (66.7 to 88.9)
pSS	36	11.1 (0.8 to 21.4)	83.3 (71.1 to 95.5)	77.8 (64.2 to 91.4)
TA	30	13.3 (1.1 to 25.4)	90 (79.3 to 100.7)	86.7 (74.5 to 98.8)
PM	28	21.4 (6.2 to 36.6)	82.1 (67.9 to 96.3)	82.1 (67.8 to 96.3)
WG	26	19.2 (4.1 to 34.3)	69.2 (51.4 to 86.9)	65.4 (47.1 to 83.7)
Control	234	11.5 (7.4 to 15.6)	82.9 (78.1 to 87.7)	76.9 (71.5 to 82.3)

Data are expressed in % (95% CI).
*p<0.05. Comparison between patients and controls
ARD, autoimmune rheumatic diseases; AS, ankylosing spondylitis; BD, Behçet's disease; DM, dermatomyositis; MCTD, mixed connective tissue disease; PAPS, primary antiphospholipid syndrome; PM, polymyositis; PsA, psoriatic arthritis; pSS, primary Sjögren's syndrome; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SSc, systemic sclerosis; TA, Takayasu's arteritis; WG, Wegener's granulomatosis.

Table 3 Geometric Mean Titre (GMT) and factor increases after influenza A (H1N1) 2009 vaccination in ARD patients and controls

		GMT		
		Before immunisation	After immunisation	
	N	Value (95% CI)		Factor increase in GMT
ARD	1668	8.0 (7.7 to 8.4)*	71.5 (66.2 to 77.3)*	8.9 (8.3 to 9.6)*
SLE	572	7.5 (7.0 to 8.0)*	59.2 (51.9 to 67.4)*	7.9 (7.0 to 8.9)*
RA	343	8.0 (7.3 to 8.8)	57.7 (48.1 to 69.1)*	7.2 (6.1 to 8.5)*
PsA	101	8.5 (7.1 to 10.1)	56.0 (40.2 to 77.9)*	6.6 (4.9 to 8.9)*
AS	152	7.9 (6.9 to 8.9)	80.0 (62.8 to 101.9)*	10.2 (8.1 to 12.8)
BD	85	7.2 (6.0 to 8.6)	95.7 (68.2 to 134.3)	13.4 (9.8 to 18.2)
DM	45	7.5 (6.0 to 9.2)	83.8 (48.3 to 145.5)	11.2 (6.8 to 18.6)
SSc	127	11.2 (9.2 to 13.7)	134.4 (101.4 to 178)	12.0 (9.1 to 15.8)
MCTD	69	8.3 (6.8 to 10.3)	83.3 (59.0 to 117.6)	10.0 (7.0 to 14.2)
PAPS	54	6.5 (5.5 to 7.5)	83.4 (57.8 to 120.4)	12.9 (9.0 to 18.6)
pSS	36	8.9 (6.4 to 12.4)	95.1 (59.4 to 152.3)	10.7 (6.6 to 17.2)
TA	30	8.5 (6.1 to 11.8)	179.6 (102.1 to 316.0)	21.1 (11.7 to 38.1)
PM	28	11.3 (7.6 to 16.9)	148.5 (86.2 to 256)	13.1 (7.9 to 21.9)
WG	26	9.2 (5.9 to 14.4)	73.9 (42.1 to 129.5)	8 (5.0 to 12.7)
Control	234	9.3 (8.2 to 10.5)	122.9 (103.4 to 146.1)	13.2 (11.1 to 15.8)

Data are expressed in value (95% CI).
*p<0.05.
ARD, autoimmune rheumatic diseases; AS, ankylosing spondylitis; BD, Behçet's disease; DM, dermatomyositis; GMT, geometric mean titre; MCTD, mixed connective tissue disease; PAPS, primary antiphospholipid syndrome; PM, polymyositis; PsA, psoriatic arthritis; pSS, primary Sjögren's syndrome; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SSc, systemic sclerosis; TA, Takayasu's arteritis; WG, Wegener's granulomatosis.

9.6 vs 13.2, 95% CI 11.1 to 15.8, p<0.0001) were significantly lower in the ARD group compared with the control group. Disease evaluations for specific patient subgroups revealed lower GMTs after immunisation for SLE (p<0.0001), RA (p<0.0001), PsA (p<0.0001) and AS (p=0.004) patients compared with healthy subjects. The increase in post-vaccination titres in GMTs was also significantly lower in SLE (p<0.0001), RA (p<0.0001) and PsA (p<0.0001) patients compared with normal controls (table 3).

Multivariate logistic regression was performed to analyse the possible influence of relevant parameters for seroconversion rates (disease (RA, SLE and PsA), seroprotection rate before immunisation, age >60 years, glucocorticoid and immunosuppressive use). Only SLE (p<0.001), RA (p<0.001) and age >60 years (p=0.004) remained significant.

Regarding disease activity, the mean SLEDAI pre-vaccination scores were similar in seroconverter and non-seroconverter SLE patients (p=0.08). Mean levels of pre-vaccination inflammatory markers were also comparable in seroconverter and non-seroconverter RA patients (ESR, p=0.11 and CRP, p=0.82).

All participants who completed the study have returned their adverse events questionnaire. No severe side effects were reported in patients and controls up to 3 weeks of follow-up. Minor local reactions were more frequently observed in controls than in patients (8.3% vs 14.1%, p=0.007), whereas the frequency of mild systemic reactions were comparable between both groups (24.6% vs 25.6%, p=0.75), with a significant higher frequency of arthralgia and fever and a lower frequency of sore throat in ARD patients compared with control group (table 4).

Table 4 Adverse events of influenza A (H1N1) 2009 vaccination in autoimmune rheumatic disease (ARD) and control patients

	ARD (n=1668)	Control (n=234)	p Value
Local reactions	139 (8.3)	33 (14.1)	0.007
Pain	105 (6)	31 (13)	0.0004
Redness	14 (0.8)	6 (2.6)	0.03
Swelling	40 (2.3)	11 (4.7)	0.05
Itching	33 (2)	1 (0.4)	0.11
Systemic reactions	411 (24.6)	60 (25.6)	0.75
Arthralgia	150 (9)	9 (3.8)	0.005
Fever	66 (3.9)	3 (1.2)	0.04
Headache	207 (12.4)	34 (14.5)	0.35
Myalgia	188 (11.3)	22 (9.4)	0.44
Sore throat	79 (4.7)	20 (8.5)	0.02
Cough	104 (6.2)	13 (5.6)	0.77
Diarrhoea	67 (4)	15 (6.4)	0.12
Rhinorrhoea	105 (6)	16 (6.8)	0.77
Nasal congestion	113 (6.8)	14 (6)	0.78

Data are expressed in N (%).

DISCUSSION

To our knowledge, this is the largest evaluation of the pandemic unadjuvanted influenza A (H1N1) 2009 virus immunisation safety in ARDs reported to date. The vaccine was generally well tolerated and had a favourable safety profile. A diverse pattern of immune responses was observed among patients with different diseases, with substandard antibody production in SLE, RA, PsA, AS, BD and DM patients.

Pre-existing immunity to the pandemic virus evaluated herein was comparable in patients and controls with an overall seroprotective rate higher than that reported in 12 691 people recruited in China.[23] The recommendation for voluntary annual seasonal flu vaccination in ARD patients with pulmonary involvement and those receiving immunosuppressive therapy may not account for this difference, as contemporary seasonal flu vaccines offer little or no advantage regarding antibody responses to the pandemic influenza A H1N1/2009 vaccine.[24]

In the present study, the non-adjuvant preparation was chosen to prevent triggering an 'adjuvant disease' in genetically susceptible individuals.[25 26] Because adjuvants may act as ligands for Toll-like receptors or stimulate innate immune responses, molecular mimicry is a potential risk in autoimmune-prone individuals. In this regard, aluminium adjuvants commonly used in

human vaccines were found to be associated with macrophagic myofasciitis, an autoimmune-related disease.[25 26] In addition, for the pandemic influenza A H1N1 vaccine tested in a large population in China, the non-adjuvanted formulation was more effective compared with adjuvant formulations with equivalent antigen content.[23]

Immunogenicity against the 2009 pandemic H1N1 influenza vaccination was significantly lower in the present group of patients with ARDs. This reduction in antibody production to viral proteins is likely associated with the impaired immune state of patients with these illnesses, as a previous study reported that T lymphocyte-dependent responses to protein antigens are compromised in these patients.[27] This feature may be an inherent characteristic of rheumatic diseases, whereas in other immunosuppressive conditions such as long-term haemodialysis[28] and renal transplant,[29] a high rate of protective immunity was observed for the seasonal flu vaccine. Alternatively, this discrepancy in renal patients may reflect frequent seasonal immunisations in previous years with vaccines of similar composition, which is not the case for the pandemic H1N1 flu vaccine.[29] Reinforcing this latter possibility, recent studies on stem cell transplantation and HIV patients reported a reduced response in these patients to the pandemic vaccine.[5–7]

Of note, the higher mean age of our patient group does not seem to explain the reduced immunogenicity observed since the subgroup analysis performed herein including patients and age-matched controls confirmed the reduced response in ARD patients. Likewise, the female predominance of our rheumatic disease patients cannot contribute to the observed decrease in humoral responses in light of previous findings that women have higher antibody titres to a large number of viral and bacterial vaccines.[30]

Importantly, attenuated protective immunogenicity against the pandemic influenza A H1N1/2009 vaccine was not a universal finding in rheumatic diseases and most likely reflects the specific immune alterations associated with therapy of each illness that ultimately affect antibody production. In this regard, this is the first and largest prospective study performed demonstrating a high 2009 pandemic H1N1 vaccine seroprotection rate, seroconversion rate and a significant increase in GMT for adults with MCTD, SSc, PAPS, pSS, PM, WG and TA. Reinforcing these findings, two recent studies with a small number of WG patients reported an adequate humoral and cellular response to the seasonal flu non-pandemic virus.[31] An elegant study on SSc patients reported effective humoral and cellular responses to an adjuvanted virosomal non-pandemic flu vaccine.[32] As for other connective tissue diseases such as MCTD, PAPS, pSS, PM and TA, no data are available regarding immune responses to flu vaccination, but the adequate seroprotection and seroconversion rates observed herein for the 2009 H1N1 flu virus may support extending the recommendation for seasonal flu vaccination in these patients.

In the present study, vaccine immune responses were significantly decreased in SLE, RA and PsA patients and reflected the low seroprotection and seroconversion rates as well as the inadequate rise in factor increase in GMT. A reduction in seroprotection with adequate seroconversion was also observed in DM, AS and BD patients. Our finding settles the controversy raised by previous reports on SLE patients, one of which reported normal immune responses in a small number of patients immunised with the seasonal flu vaccine[33–37] and another that reported reduced immune responses in patients immunised with the 2009 H1N1 pandemic vaccine.[8] This reduced immunogenicity is likely due to impairments in humoral and cellular immunity in SLE patients that may ultimately affect the response to antigenic challenge.[37] The unexpectedly low immune responses in RA patients contrast with previous studies, including two with randomised designs reporting normal immunogenicity to the seasonal vaccine in RA patients.[38 39] The use of non-adjuvanted vaccine in the present study may partly explain this discrepancy, as the use of adjuvants may be required in RA to achieve maximum immunogenicity.[39]

Alternatively, glucocorticoid and immunosuppressive drugs may account for the deficient vaccine responses in rheumatic disease patients. However, several reports have demonstrated that, with the exception of rituximab,[40 41] glucocorticoids, methotrexate and tumour necrosis factor blockers do not have a deleterious effect on the immunogenicity of the seasonal flu vaccine in RA[37 38 42 43] and AS.[43] The small number of patients using rituximab in our group of patients (0.8%) cannot explain the deficient immune responses observed in some rheumatologic disease patients.

It is important to emphasise that immunisation had an excellent safety profile and that only mild reactions were observed. The possibility of autoantibodies produced post-vaccination, the exacerbation of established rheumatic diseases and the influence of therapy[38 44] are currently being evaluated in different subgroups and will be the subject of another publication. Importantly, neurological autoimmune diseases following flu immunisation, such as Guillain–Barré syndrome, acute encephalomyelitis or transverse myelitis,[44] were not observed in this population, which is theoretically more prone to develop autoimmunity. We cannot exclude, however, the possibility that a severe side effect might have been missed since not all participants returned to the second phase. This possibility is unlikely since all patients are still followed in our Outpatient Rheumatology Clinic.

In summary, this is the largest prospective study of pandemic unadjuvanted influenza A (H1N1) 2009 virus immunisation in ARDs patients that provides clear evidence of its safety. A distinct disease profile of immunogenicity was identified, and further studies are necessary to determine if a booster dose will be effective for those with suboptimal immune responses.

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Original article

Influenza A/H1N1 vaccination of patients with SLE: can antimalarial drugs restore diminished response under immunosuppressive therapy?

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Abstract

Objective. To assess the efficacy and safety of pandemic 2009 influenza A (H1N1) in SLE under different therapeutic regimens.

Methods. A total of 555 SLE patients and 170 healthy controls were vaccinated with a single dose of a non-adjuvanted preparation. According to current therapy, patients were initially classified as SLE No Therapy ($n = 75$) and SLE with Therapy ($n = 480$). Subsequent evaluations included groups under monotherapy: chloroquine (CQ) ($n = 105$), prednisone (PRED) $\geqslant 20$ mg ($n = 76$), immunosuppressor (IS) ($n = 95$) and those with a combination of these drugs. Anti-H1N1 titres and seroconversion (SC) rate were evaluated at entry and 21 days post-vaccination.

Results. The SLE with Therapy group had lower SC compared with healthy controls (59.0 *vs* 80.0%; $P < 0.0001$), whereas the SLE No Therapy group had equivalent SC (72 *vs* 80.0%; $P = 0.18$) compared with healthy controls. Further comparison revealed that the SC of SLE No Therapy (72%) was similar to the CQ group (69.5%; $P = 0.75$), but it was significantly reduced in PRED $\geqslant 20$ mg (53.9%; $P = 0.028$), IS (55.7%; $P = 0.035$) and PRED $\geqslant 20$ mg + IS (45.4%; $P = 0.038$). The concomitant use of CQ in each of these later regimens was associated with SC responses comparable with SLE No Therapy group (72%): PRED $\geqslant 20$ mg + CQ (71.4%; $P = 1.00$), IS + CQ (65.2%; $P = 0.54$) and PRED $\geqslant 20$ mg + IS + CQ (57.4%; $P = 0.09$).

Conclusion. Pandemic influenza A H1N1/2009 vaccine response is diminished in SLE under immunosuppressive therapy and antimalarials seems to restore this immunogenicity.

Trial registration. www.clinicaltrials.gov, NCT01151644.

Key words: systemic lupus erythematosus, vaccine, infection, influenza, antimalarials, disease-modifying anti-rheumatic drugs, immunosuppressive, H1N1 vaccination, immune response, efficacy, prevention.

Introduction

Infections in SLE are considered to be important causes of morbidity/mortality [1–3], and vaccination is the most effective preventive measure to control virus dissemination and to reduce associated complications [4].

Pandemic influenza is of particular concern for these immunosuppressed patients due to its additional catastrophic nature, as was reported for the influenza H1N1 (Spanish flu, 1918), A/H2N2 (Asian influenza, 1957),

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A/H3N2 (Hong Kong flu, 1968) and H1N1 (Swine flu, 1976; Russian flu, 1977) strains [5]. In 2009, a new pandemic A/California/7/2009 (H1N1) virus emerged, requiring major public health efforts [6]. Its spread throughout the world prompted the decision that the Northern Hemisphere's 2010–11 and the Southern Hemisphere's 2011 trivalent seasonal influenza vaccines must contain the A/California/7/2009 (H1N1)-like virus [7]. Immunocompromised patients have indications to receive this vaccine according to the European League Against Rheumatism [8, 9] and the 2010 Recommendations of the Advisory Committee on Immunization Practices [10], but the role of therapy and the vaccine's immunogenicity in SLE are still unclear.

Vaccine immune response in SLE may be subdued as a consequence of immunological changes intrinsic to this immune-mediated disease [8, 9, 11]. Therefore the inclusion of a sizeable number of lupus patients not receiving therapy seems to be a necessary condition to appropriately define the influence of the disease on immunogenicity, a condition not met by previous studies [12–21]. In addition, the concomitant use of multiple therapies is a common feature of SLE, which reinforces the need for an adequate population to determine the effect of drugs on the pandemic influenza A H1N1/2009 vaccine response to avoid the pitfalls of subgroup analysis. In fact, the anti-H1N1 influenza serum antibody response in SLE patients under different immunosuppressive drugs is still controversial [8, 11], with some evidence of decreased efficacy for AZA [13, 16, 20] and glucocorticoids [14, 16, 17] and scarce data for other commonly used drugs [12, 14, 18, 19].

With regard to chloroquine (CQ), this drug was recently suggested as a promising candidate to improve immune response to vaccines [22, 23]. Indeed, it has been demonstrated that treatment with CQ improves primary $CD8^+$ T-cell stimulation by soluble ovalbumin in experimental models [24] and enhances human memory $CD8^+$ T-cell response against HBV antigens [25]. This improved cellular immune response may ultimately increase antibody production through more efficient support for B cells, as previously demonstrated for virosomal vaccines [26]. Reinforcing this possibility, an increase in meningococcal and diphtheria vaccine responses was observed in individuals under CQ chemoprophylaxis [27–29].

In SLE, the only three studies evaluating the influence of HCQ in seasonal [15] and pandemic [20, 30] influenza immunization revealed an effective response, but the very limited samples evaluated preclude a definitive conclusion about their findings. The present report was therefore designed to prospectively assess the impact of lupus disease and therapy in the pandemic influenza A H1N1/2009 vaccine response in a large SLE cohort.

Patients and methods

This study initially selected 638 lupus patients who were included in a large, prospective rheumatic disease cohort conducted at a single site in São Paulo, Brazil (Rheumatology Division, Hospital das Clínicas da Universidade de São Paulo), between March and April 2010, and who completed the study, described in detail elsewhere [31]. The study was approved by the local institutional review board (Comissão de Ética para Análise de Projetos de Pesquisa—CAPPesq HCMUSP, #114/10), and all participants signed the informed consent. The trial was registered at clinicaltrials.gov under NCT01151644.

Patients

Adult ($\geqslant$18-year-old) SLE patients (ACR criteria) [32] regularly followed at the out-patient Lupus Clinic, Rheumatology Division of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, were initially recruited by letter to participate in the Public Health influenza A H1N1/2009 vaccine campaign at the immunization centre of our hospital.

The exclusion criteria were previous known infection with influenza A (H1N1) 2009, an anaphylactic response to vaccine components or to eggs, an acute infection resulting in a fever of $>38^{\circ}$C at the time of vaccination, a history of Guillain–Barré syndrome or demyelination syndromes, previous vaccination with any live vaccine 4 weeks before the study or any inactivated vaccine 2 weeks before the study, a 2010 seasonal influenza vaccination, a blood transfusion within 6 months, hospitalization or failure to complete the protocol.

The study included two phases: entry and a follow-up period of 21 days with a diary card. A clinical and laboratory evaluation was performed, including disease activity parameters according to the SLEDAI [33], and a blood sample was obtained from each participant immediately before vaccination and 21 days after vaccination.

Five hundred and fifty-five patients fulfilled the inclusion criteria and completed the two study phases. Their charts were extensively reviewed for additional clinical and treatment data. SLE manifestations were defined as cutaneous disease (malar or discoid rash, oral ulcers or photosensitivity), articular involvement (arthralgia or non-erosive arthritis involving two or more peripheral joints), neuropsychiatric disease (psychosis, depression, seizure or peripheral neuropathy), renal disease (persistent proteinuria >0.5 g/24 h, presence of cellular casts of red or white blood cells or mixed, persistent haematuria >10 red blood cells per high power field and five leucocytes per high power field, excluding infection or stones), cardiopulmonary disease (serositis, myocarditis, restrictive lung disease or pulmonary hypertension) and haematological complications (haemolytic anaemia, leucopenia with a white blood cell count $<4000/mm^3$, lymphopenia $<1500/mm^3$ on two or more occasions or thrombocytopenia with a platelet count $<100\,000/mm^3$ in the absence of drugs).

Controls

One hundred and seventy age- and sex-matched healthy subjects who came to this centre seeking vaccination in response to a Public Health national campaign were invited to participate as a control group with the same

exclusion criteria. These individuals were selected from the previously described large study of the H1N1 vaccine immune response in rheumatic diseases [31].

Vaccine

The H1N1 vaccine, a novel, monovalent, unadjuvanted, inactivated and split-virus vaccine, was produced by Butantan Institute/Sanofi Pasteur (São Paulo, Brazil). The active substance is a split, inactivated influenza virus containing antigens equivalent to the A/California/7/2009 (H1N1) virus-like strain (NYMCx-179A), one of the candidate reassortant vaccine viruses recommended by the WHO. The vaccine was prepared in embryonated chicken eggs, with the same standard techniques that are used for the production of seasonal trivalent inactivated vaccines, and it was presented in 5-ml multidose vials, with thimerosal added as a preservative (45 μg/0.5 ml dose).

Study procedures

All subjects were vaccinated with the pandemic H1N1 influenza vaccine (A/California/7/2009, Butantan Institute/Sanofi Pasteur). A single i.m. dose (0.5 ml) of 15 μg haemagglutinin antigen, specific for the A/California/7/2009 (H1N1)-like virus, was administered.

Safety assessments

A 21-day diary card was given to each participant at entry with 13 (yes or no) established reactions. This written card included local reactions (pain, redness, swelling and itching) and systemic adverse events such as arthralgia, fever, headache, myalgia, sore throat, cough, diarrhoea, rhinorrhoea and nasal congestion. Participants were required to return their diary cards at the end of the follow-up period (21 days after vaccination). All local reactions were considered to be related to the H1N1 vaccine. Recorded symptoms were checked by the investigators to determine the causality of solicited systemic adverse events, and unsolicited adverse events were also assessed. Severe side effects were defined as those requiring hospitalization or leading to death.

Laboratory assays

Blood samples were collected at baseline and 3 weeks after vaccination, and sera were stored at −70°C. The two samples from each patient or control were tested in parallel in the same plate for all laboratory determinations. The immunogenicity of the A/California/7/2009 (H1N1)-like virus vaccine was evaluated with the use of a haemagglutination inhibition assay (HIA) at the Adolfo Lutz Institute.

HIA

The influenza virus antigen used in this study was the A/California/7/2009 (H1N1), supplied by the Butantan Institute. Virus concentrations were determined by haemagglutinin antigen titration, and the HIA test was performed after removing naturally occurring, non-specific inhibitors from the sera, as previously described [34]. The H1N1 vaccination immunoresponse was evaluated by determining the levels of antibodies by haemagglutination inhibition. Anti-H1N1 titre was determined by influenza HIA. The percentages of seroprotection (SP) (titre $\geqslant 1:40$) and seroconversion (SC) (a pre-vaccination titre $<1:10$ and a post-vaccination HIA titre $\geqslant 1:40$ or pre-vaccination titres $\geqslant 1:10$ and a $\geqslant 4$-fold rise post-vaccination), geometric mean titres (GMTs) and factor increases (FIs) in GMTs were calculated.

Serological determinations

Serum samples were stored at −70°C until use. Anti-dsDNA antibody titres were detected by ELISA with purified antigen using a commercially available kit (INOVA Diagnostics Inc., San Diego, CA, USA). Serum levels of C3 complement fractions were measured by RID (SIEMENS Health Care, Marburg, Germany).

Statistical analysis

The sample size was chosen practically rather than statistically because of the need to obtain robust estimates of vaccine immunogenicity in lupus patients. The large sample size of the lupus population and controls gave the study a power analysis >80%.

The analyses were descriptive, with the calculation of two-sided 95% CIs, assuming binomial distributions for dichotomous variables and a log-normal distribution for haemagglutination inhibition titres. For categorical variables, statistical summaries included the rates of SC and SP; these rates were compared using Fisher's exact test. Every subgroup had its haemagglutination inhibition GMT calculated before vaccination and 21 days after vaccination. The FI in GMT (i.e. the ratio of the titres after vaccination to the titres before vaccination) was also obtained. Log-transformed FI was compared between subgroups of SLE patients using a two-sided Student's *t*-test with an α-level of 0.05.

Results

Among the 638 lupus patients initially vaccinated, 555 (87%) completed the study and comprised the patient group. SLE patients and controls had similar ages [36.7 (12.2) *vs* 38.7 (13.2) years old; $P = 0.28$] and frequencies of female sex (92.6 *vs* 90.6%; $P = 0.41$). The mean disease duration was 13.0 (8.9) years, and the frequencies of current/previous SLE manifestations were cutaneous (67.9%), articular (60.5%), neuropsychiatric (13.9%), renal (41.4%), cardiopulmonary (16.6%) and haematological (33.5%).

At entry, the overall analysis of patients' therapies revealed 75 (13.5%) without drugs; 350 (63%) under CQ diphosphate (all using 250 mg/day), with 105 as monotherapy (18.9%); 303 under glucocorticoids (54.6%), with a current mean prednisone (PRED) dose of 7.7 (11.3) mg/day, 16% with doses >20 mg/day and 3.8% with doses >40 mg/day; and 286 (51.5%) under immunosuppressors (ISs) with the following distribution among them: 115 (20.7%) AZA, 87 (15.7%) MMF, 65 (11.7%) MTX and 19 (3.4%) i.v. CYC. Exclusive use of ISs was

identified in 95 patients: 38 (6.8%) AZA [mean dose of 127.0 (50.1) mg/day], 30 (5.4%) MMF [mean dose of 2.26 (0.85) g/day] and 27 (4.9%) MTX [mean dose of 16.4 (5.7) mg/week].

Vaccine immunoresponse

Before immunization, SP rates were comparable in the SLE group and healthy controls ($P = 0.36$). Three weeks after vaccination, significantly lower SP [64.7% (95% CI 60.7%, 68.7%) *vs* 84.1% (95% CI 78.6%, 89.6%); $P < 0.0001$] and SC rates [60.7% (95% CI 56.7%, 64.8%) *vs* 80.0% (95% CI 74.0%, 86.0%); $P < 0.0001$] were observed in SLE compared with the healthy controls. The FI in GMTs after immunization were significantly lower in SLE compared with the healthy control group [8.0 (95% CI 7.1, 9.0) *vs* 14.4 (95% CI 11.7, 17.6); $P < 0.0001$].

Effect of disease and therapy on immune response

SLE without any therapy (the SLE No Therapy group) had equivalent post-vaccine SP [74.7% (95% CI 64.8%, 84.5%) *vs* 84.1% (95% CI 78.6%, 89.6%); $P = 0.10$] and SC rates [72.0% (95% CI 61.8%, 82.1%) *vs* 80.0% (95% CI 74.0%, 86.0%); $P = 0.18$] compared with the healthy control group (Table 1). In contrast, the SLE with Therapy group had significantly lower SP [63.1% (95% CI 58.8%, 67.4%) *vs* 84.1% (95% CI 78.6%, 89.6%); $P < 0.0001$] and SC rates [59.0% (95% CI 54.6%, 63.4%) *vs* 80.0% (95% CI 74.0%, 86.0%); $P < 0.001$] compared with controls (Table 1). The FI in GMTs of the SLE No Therapy group was comparable with the healthy control group [11.7 (95% CI 8.6, 15.9) *vs* 14.4 (95% CI 11.7, 17.6); $P = 0.28$], whereas the SLE with Therapy group had significantly lower levels [7.6 (95% CI 6.6, 8.6); $P < 0.0001$].

The comparison of the SLE No Therapy and SLE with Therapy groups revealed a significantly higher SC rate ($P = 0.032$) and a trend towards a higher SP rate ($P = 0.052$) in the former group (Table 1). Likewise, a lower FI in GMTs was detected in the SLE with Therapy group compared with SLE No Therapy ($P = 0.013$). The SLE No Therapy group had significantly lower mean SLEDAI scores [2.2 (3.5) *vs* 3.3 (4.0); $P = 0.0042$] and higher mean lymphocyte counts [1628 (759) *vs* 1407 (723)/mm^3; $P = 0.007$] compared with the SLE with Therapy group, but they had similar mean leucocyte counts [5797 (2338) *vs* 6142 (2632)/mm^3; $P = 0.34$].

Of note, SLE patients with CQ monotherapy had similar post-vaccination SP [78.0% (95% CI 70.1%, 85.9%); $P = 0.60$] and SC [69.5% (95% CI 60.7%, 78.3%); $P = 0.75$] rates compared with the SLE No Therapy group and significantly higher SP rates before immunization ($P = 0.0076$) (Table 2). FIs in GMTs were comparable in the CQ and SLE No Therapy groups [8.8 (95% CI 7.0, 11.2) *vs* 11.7 (95% CI 8.6, 15.9); $P = 0.21$). These two groups had similar mean SLEDAI scores [2.8 (3.4) *vs* 2.2 (3.5); $P = 0.11$], mean lymphocyte counts [1417 (665) *vs* 1628 (759)/mm^3; $P = 0.45$] and mean leucocyte counts [6505 (2961) *vs* 5797 (2338)/mm^3; $P = 0.22$].

SLE patients using steroids [mean PRED dose of 14.1 (12.0) mg/day] had a significantly lower SC rate after vaccination ($P = 0.04$) and FI in GMT [7.3 (95% CI 5.5, 9.6); $P = 0.04$] and a trend towards a lower SP rate ($P = 0.051$) compared with the SLE No Therapy group (Table 2). A significantly lower SC rate [53.9% (95% CI 42.7%, 65.1%); $P = 0.028$] and a reduced FI in GMT [6.7 (95% CI 4.8, 9.4); $P = 0.018$] were observed in SLE patients using >20 mg/day of PRED compared with the SLE No Therapy group. In contrast, the concomitant use of PRED $\geqslant 20$ mg/day and CQ (PRED $>20 + $ CQ) resulted in a comparable SC rate [71.4% (95% CI 47.7%, 95.0%); $P = 1.00$] (Table 2) and FI in GMT [8.8 (95% CI 6.2, 12.5); $P = 0.49$] compared with that observed in the SLE No Therapy group. These two groups of patients (PRED >20 and PRED $>20 + $ CQ) had comparable mean PRED doses [27.8 (10.6) *vs* 24.3 (7.5) mg/day; $P = 0.25$], mean SLEDAI scores [2.9 (3.7) *vs* 4.1 (6.2); $P = 0.90$], mean lymphocyte counts [1485 (697) *vs* 1050 (475)/mm^3; $P = 0.11$] and mean leucocyte counts [6195 (3173) *vs* 4741 (1970)/mm^3; $P = 0.11$].

SLE patients using ISs had significantly lower SC [55.7% (95% CI 45.7%, 65.7%); $P = 0.035$], SP [58.9% (95% CI 49.0%, 68.8%); $P = 0.037$] and FI in GMT [7.2 (95% CI 5.4, 9.5); $P = 0.041$] after vaccination compared with the SLE No Therapy group (Table 2). Further analysis revealed that SLE patients with AZA had a significantly lower SC rate ($P = 0.024$) and a trend towards a lower SP rate after vaccination ($P = 0.053$) compared with the SLE No Therapy group (Table 2). MTX also had a reduced SC rate ($P = 0.03$) and a trend towards a lower SP rate after vaccination ($P = 0.051$) compared with the SLE No

Table 1 SP and SC rates of influenza A (H1N1) 2009 vaccine in SLE and controls

Group	*n*	SP rate (titre $\geqslant$1/40), % (95% CI)		SC rate, % (95% CI)
		Before immunization	After immunization	
SLE	555	8.6 (6.3, 11.0)	64.7 (60.7, 68.7)*	60.7 (56.7, 64.8)*
SLE with Therapy	480	9.2 (6.6, 11.8)	63.1 (58.8, 67.4)*	59.0 (54.6, 63.4)*,**
SLE No Therapy	75	5.3 (0.3, 10.4)	74.7 (64.8, 84.5)	72.0 (61.8, 82.1)
Control	170	11.2 (6.4, 15.9)	84.1 (78.6, 89.6)	80.0 (74.0, 86.0)

*$P < 0.0001$ *vs* control; **$P < 0.05$ *vs* SLE No Therapy.

Table 2 SP and SC rates of influenza A (H1N1) 2009 vaccine in systemic lupus patients according to therapy

Group	*n*	SP rate (titre ⩾1/40), % (95% CI)		SC rate, % (95% CI)
		Before immunization	After immunization	
SLE No Therapy	75	5.3 (0.3, 10.4)	74.7 (64.8, 84.5)	72.0 (61.8, 82.1)
CQ	105	19.0 (11.5, 26.5)*	78.0 (70.1, 85.9)	69.5 (60.7, 78.3)
PRED	99	8.8 (2.6, 13.3)	59.6 (49.9, 69.2)	56.6 (46.8, 66.3)*
PRED ⩾20	76	7.9 (1.8, 13.9)	63.1 (52.2, 73.9)	53.9 (42.7, 65.1)*
PRED ⩾20 + CQ	14	7.1 (−6.3, 20.6)	71.4 (47.7, 95.0)	71.4 (47.7, 95.0)
IS	95	3.0 (−0.4, 6.4)	58.9 (49.0, 68.8)*	55.7 (45.7, 65.7)*
AZA	38	2.6 (−2.4, 7.6)	55.2 (39.4, 71.0)	50.0 (34.1, 65.9)*
MTX	27	3.7 (−3.4, 10.8)	51.8 (32.9, 70.6)	48.1 (29.2, 66.9)*
MMF	30	3.3 (−3.0, 9.7)	46.6 (28.7, 64.4)*	40.0 (22.5, 57.5)*
IS + CQ	46	6.5 (−0.6, 13.6)	67.4 (53.8, 80.9)	65.2 (51.5, 79.0)
PRED ⩾20 + IS	22	4.5 (−4.1, 13.1)	59.1 (38.6, 79.6)	45.4 (24.6, 66.2)*
PRED ⩾20 + IS + CQ	54	5.5 (−0.6, 11.6)	63.0 (50.1, 75.9)	57.4 (44.2, 70.6)

*$P < 0.05$ *vs* SLE No Therapy.

Therapy group (Table 2). MMF induced a significantly lower SC rate ($P = 0.003$) and SP rate after vaccination ($P = 0.01$) compared with the SLE No Therapy group. The association of ISs and CQ (IS + CQ) disclosed similar SC [65.2% (95% CI 51.5%, 79.0%); $P = 0.54$], SP [67.4% (95% CI 53.8%, 80.9%); $P = 0.41$] and FI in GMT [9.9 (95% CI 6.6%, 14.7%); $P = 0.58$] rates to those found in the SLE No Therapy group (Table 2). A higher mean SLEDAI score was identified in IS + CQ compared with ISs [3.6 (3.8) *vs* 2.6 (3.6); $P = 0.034$], but the mean lymphocyte count [1525 (590) *vs* 1339 (624)/mm^3; $P = 0.41$] and mean leucocyte count [7210 (1056) *vs* 5754 (2399)/mm^3; $P = 0.55$] were alike among groups.

Nineteen SLE patients were using CYC, but the majority ($n = 16$) were also using CQ diphosphate. The apparent low SP [57.9 (95% CI 35.7, 80.1)] and SC [57.9 (95% CI 35.7, 80.1)] rates observed in SLE patients with CYC did not reach statistical significance compared with the SLE No Therapy group ($P = 0.17$ and $P = 0.27$, respectively).

Patients under the combination of PRED >20 mg/day and ISs (PRED>20 + IS) had a significantly lower SC rate [45.4% (95% CI 24.6, 66.2); $P = 0.038$] and FI in GMT [6.1 (95% CI 4.3, 8.7); $P = 0.048$] compared with those of the SLE No Therapy group (Table 2). The same combination with concomitant use of CQ (PRED>20 + IS + CQ) achieved an SC rate [57.4% (95% CI 44.2%, 70.6%); $P = 0.09$] and FI in GMT [7.8 (95% CI 5.6, 10.9); $P = 0.10$] similar to the SLE No Therapy group (Table 2). The two groups of patients (PRED>20 + IS and PRED>20 + IS + CQ) had comparable mean PRED doses [27.5 (9.5) *vs* 28.3 (10.9) mg/day; $P = 0.86$], mean SLEDAI scores [3.3 (3.3) *vs* 2.8 (3.9); $P = 0.29$], mean lymphocyte counts [1441 (701) *vs* 1370 (719)/mm^3; $P = 0.59$] and mean leucocyte counts [6125 (2402) *vs* 6036 (2967)/mm^3; $P = 0.63$].

Safety

Regarding lupus safety, no significant differences were observed among SLEDAI scores before and 3 weeks after immunization [3.2 (3.9) *vs* 2.8 (3.2); $P = 0.62$], and no patients had new major organ involvement. The frequency of positive anti-dsDNA (47.2 *vs* 44%; $P = 0.33$) and the mean C3 levels [100.5 (30.5) *vs* 100.3 (30.8) mg/dl; $P = 0.70$] were comparable before and after vaccination.

No severe side effects were reported in any groups over the 3 weeks of follow-up. Minor local reactions (8.6 *vs* 17.6%; $P = 0.017$) and mild systemic reactions (18.3 *vs* 27.6%; $P = 0.012$) were less frequently observed in SLE than in controls, in particular, influenza-related symptoms such as headache ($P = 0.013$), myalgia ($P = 0.015$), sore throat ($P = 0.0008$), rhinorrhoea ($P = 0.0016$) and nasal congestion ($P = 0.027$) (Table 3). No severe side effects were observed in SLE patients or controls.

Table 3 Adverse events of influenza A (H1N1) 2009 vaccine in SLE and controls

Parameter	SLE ($n = 555$)	Control ($n = 170$)	*P*
Local reactions	48 (8.6)	30 (17.6)	0.017
Pain	43 (7.7)	28 (16.4)	0.017
Redness	14 (2.5)	6 (3.5)	0.43
Swelling	18 (3.2)	11 (6.4)	0.07
Itching	8 (1.4)	1 (0.6)	0.69
Systemic reactions	102 (18.3)	47 (27.6)	0.012
Fever	15 (2.7)	3 (1.7)	0.77
Arthralgia	22 (3.9)	9 (5.3)	0.51
Headache	55 (9.9)	29 (17)	0.013
Myalgia	31 (5.5)	19 (11.2)	0.015
Diarrhoea	15 (2.7)	12 (7)	0.017
Sore throat	18 (3.2)	17 (10)	0.0008
Cough	18 (3.2)	11 (6.4)	0.072
Rhinorrhoea	13 (2.3)	14 (8.2)	0.0016
Nasal congestion	14 (2.5)	11 (6.4)	0.027

Data are expressed as *n* (%).

Discussion

This is the first study to demonstrate that CQ counterbalances the deleterious effects of immunosuppressive therapies in lupus influenza A/H1N1 immunoresponse. Despite prevailing concerns about the risks of influenza in patients with autoimmune rheumatic diseases, including SLE, and especially patients under immunosuppression, their response to vaccine is not well determined. In fact, influenza vaccination is widely recommended for patients who are immunosuppressed, such as those with SLE (European League Against Rheumatism and 2010 Recommendations of the Advisory Committee on Immunization Practices) [8–10], but also for patients infected with HIV [35] and recipients of solid organ [36] and haemopoietic stem-cell transplants [37]. However, the same immune dysfunctions that can increase the risks and consequences of influenza infection can also compromise vaccine responses and effectiveness.

The inclusion of a sizeable number of lupus patients without any therapy and of healthy controls in the present study was an essential step in determining that the disease itself does not seem to reduce the vaccine immune response, a condition that had not been met by previous reports [12–21]. However, the observed significant decrease in SP and SC of the influenza A/H1N1 vaccine in lupus patients with therapy compared with those without drugs provides clear evidence for the deleterious role of treatment in this vaccine's antibody production.

In real-life circumstances, most lupus patients are under immunosuppression, particularly with CSs. This therapy promotes the impairment of antigen processing and has implications for the efficacy of anti-viral vaccines [38, 39], including the reduced vaccine response observed in SLE [14, 16, 17]. Regarding seasonal influenza vaccination, five studies have demonstrated impaired response [12, 13, 16, 19, 40], mainly associated with PRED doses >20 mg/day [12, 16]. According to these findings, the British Society of Rheumatology Clinical Affairs Committee [41] and the 2010 Advisory Committee on Immunization Practices [10] recommended vaccination in patients under this dose. We have confirmed and extended this observation for pandemic influenza A H1N1/2009.

With regard to the influence of ISs on seasonal influenza vaccination, AZA has been described as reducing this response, despite most patients having achieved protective levels of antibodies [13, 16, 42]. This impairment of humoral response with AZA was also observed herein and confirms the report of SLE with pandemic A H1N1/2009 influenza vaccine [20]. Our study introduces MMF as another IS that significantly reduces SP and SC in SLE patients, as is also reported for other non-rheumatological immunosuppressive conditions [43–46]. Moreover, the present study offers the first evidence in SLE that MTX negatively influences the pandemic influenza A H1N1/2009 immune response, in contrast to that observed for the seasonal influenza vaccination [16, 40]. Finally, the small representation of patients under CYC evaluated herein precluded a definitive conclusion about its possible deleterious effect on vaccination. It should be emphasized that although the vaccine response was diminished in patients with immunosuppressive drugs, the majority of these patients still have a response. Therefore the findings presented here still underline the recommendations to vaccinate all patients treated with immunosuppressive drugs.

Remarkably, the concomitant use of CQ enhanced antibody production to the pandemic influenza A H1N1/2009 vaccine in patients under PRED $\geqslant 20$ mg and/or immunosuppression. This novel beneficial effect of this drug is further supported by the observations that the mean glucocorticoid dose was comparable in these groups of patients with or without concomitant CQ therapy. Moreover, lower systemic inflammation does not seem to account for these favourable findings in view of the fact that comparable SLEDAI scores were detected among glucocorticoid or immunosuppressive groups with and without concomitant use of CQ. The mean lymphocyte count was also similar in these groups of patients with and without concurrent CQ therapy, despite previous reports that lymphopenia may influence the SC rate of the pandemic (H1N1) vaccine [47].

This finding is unique because there are no studies regarding the influenza vaccine antibody response in individuals under CQ and there are limited data for other immunizations [27–29, 48, 49]. In Nigerian children, continuous CQ chemoprophylaxis enhanced the response to meningococcal vaccines and had no depressive effects for tetanus, diphtheria, measles, poliomyelitis, typhoid or BCG [27, 28]. However, reports of reduced antibody responses to rabies, cholera and typhoid are most likely explained by their short-term simultaneous administration [48, 49]. Reinforcing the beneficial effects of CQ, an improved humoral response was reported for diphtheria vaccination (diphtheria and tetanus) with prolonged antimalarial prophylaxis [29].

As CQ treatment in lupus is usually long term, we hypothesize that the underlying mechanisms for a better immune response to the pandemic influenza H1N1/2009 vaccine associated with antimalarial therapy may be related to more efficacious generation or to the maintenance of immunological memory. In fact, we have observed a significantly higher SP rate before immunization in patients under antimalarial monotherapy compared with those without any therapy, raising the possibility of a CQ T-cell-driven broader spectrum or of a longer protection response after exposure to the first wave of the pandemic H1N1/2009 during the previous year. A recent study demonstrated that cross-presentation of soluble HBV antigens to specific $CD8^+$ T memory cell clones was dramatically improved with CQ in hepatitis vaccine boosters [25].

The effect of CQ observed herein most likely can be extended to HCQ therapy in view of the fact that SP after pandemic A H1N1/2009 influenza vaccination in a limited number of SLE patients under this drug was suggested to meet the European Committee for Proprietary Medicinal Products criteria [20].

Regarding the short-term safety of the A/California/7/2009 (H1N1)-like virus vaccine in SLE patients, adverse effects were mild and occurred at lower frequencies compared with healthy controls. None of the SLE patients developed any major neurological manifestations, such as Guillain–Barré syndrome, convulsions, psychosis or organic brain syndromes [50]. Moreover, no significant differences were observed between SLEDAI scores and the frequencies of positive anti-dsDNA before or 3 weeks after immunization. This absence of severe side effects is probably not explained by the exclusion of participants who did not return for the second phase because all patients are still being regularly followed in our Outpatient Rheumatology Clinic, but it does not exclude the possibility that a longer observation period will reveal additional vaccine or disease-related effects.

We conclude that CQ is a promising candidate to improve the pandemic influenza A H1N1/2009 immune response in SLE, including in patients under glucocorticoid and/or immunosuppressive therapy. Further studies are necessary to determine the underlying mechanism of this antimalarial adjuvant effect and its possible use as an effective strategy for other vaccines in rheumatic diseases.

Rheumatology key messages

- Antimalarials improve pandemic influenza A H1N1/2009 response in lupus patients under immunosuppressive therapy.
- SLE patients with other therapy had lower SP and SC rates compared with healthy population.
- Concomitant use of CQ enhanced antibody response even in SLE patients under PRED and/or ISs.

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Original article

TNF blockers show distinct patterns of immune response to the pandemic influenza A H1N1 vaccine in inflammatory arthritis patients

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Abstract

Objective. To evaluate the immunogenicity of the anti-influenza A H1N1/2009 vaccine in RA and spondyloarthritis (SpA) patients receiving distinct classes of anti-TNF agents compared with patients receiving DMARDs and healthy controls.

Methods. One hundred and twenty patients (RA, $n = 41$; AS, $n = 57$; PsA, $n = 22$) on anti-TNF agents (monoclonal, $n = 94$; soluble receptor, $n = 26$) were compared with 116 inflammatory arthritis patients under DMARDs and 117 healthy controls. Seroprotection, seroconversion (SC), geometric mean titre, factor increase in geometric mean titre and adverse events were evaluated 21 days after vaccination.

Results. After immunization, SC rates (58.2% *vs* 74.3%, $P = 0.017$) were significantly lower in SpA patients receiving anti-TNF therapy, whereas no difference was observed in RA patients receiving this therapy compared with healthy controls ($P = 0.067$). SpA patients receiving mAbs (infliximab/adalimumab) had a significantly lower SC rate compared with healthy controls (51.6% *vs* 74.3%, $P = 0.002$) or those on DMARDs (51.6% *vs* 74.7%, $P = 0.005$), whereas no difference was observed for patients on etanercept (86.7% *vs* 74.3%, $P = 0.091$). Further analysis of non-seroconverting and seroconverting SpA patients revealed that the former group had a higher mean age ($P = 0.003$), a higher frequency of anti-TNF ($P = 0.031$) and mAbs ($P = 0.001$) and a lower frequency of MTX ($P = 0.028$). In multivariate logistic regression, only older age ($P = 0.015$) and mAb treatment ($P = 0.023$) remained significant factors for non-SC in SpA patients.

Conclusion. This study revealed a distinct disease pattern of immune response to the pandemic influenza vaccine in inflammatory arthritis patients receiving anti-TNF agents, illustrated by a reduced immunogenicity solely in SpA patients using mAbs.

Trial Registration: ClinicalTrials.gov, www.clinicaltrials.gov, NCT01151644.

Key words: vaccine, safety, immunogenicity, pandemic influenza A (H1N1), biologic agents, rheumatic disease, TNF blockers.

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Introduction

People suffering with autoimmune rheumatic diseases (ARDs) who are treated with DMARDs [1–3] and biologic agents are recognized to be at increased risk of infection [4]. This insight was particularly relevant for the recent 2009 influenza A H1N1 pandemic, which led to a high frequency of hospitalization and death in this particular group of patients [5].

After the H1N1 A/California/7/2009 influenza pandemic, the vaccine was largely produced through immunization programs [5, 6], and both the European League Against Rheumatism [4] and the Centers for Disease Control and Prevention [5] strongly recommended that inactivated pandemic influenza vaccination should be indicated for ARD patients.

We recently studied the immunogenicity and safety of a non-adjuvanted pandemic 2009 influenza A H1N1 vaccine in 1664 ARD patients and 234 healthy controls, showing an overall reduced immune response [7]. We also observed reduced seroconversion (SC) rates in RA patients linked to MTX therapy and unrelated to disease activity [8]. Simultaneously, two studies with an adjuvanted pandemic 2009 influenza A H1N1 vaccine were published: one associated increasing age with DMARD therapy but not with anti-TNF blockers, which were associated with a low antibody response in ARD patients [9]; the second study found reduced immunogenicity in patients with RA or PsA and those on infliximab or LEF [10].

However, the limited number of subjects receiving different TNF blockers and the inclusion of diverse diseases may hamper the interpretation of these study findings because vaccine antibody response varies among the rheumatic diseases [7]. Moreover, the discrimination of the possible deleterious effects of biologic therapy on the vaccine immune response requires an evaluation of patients solely on DMARDs due to the widespread concomitant use of these drugs with biologic therapy [11].

Therefore the objective of the present study was to evaluate the immunogenicity and short-term safety of the anti-pandemic 2009 influenza A H1N1 vaccine in RA and spondyloarthritis (SpA) receiving distinct classes of anti-TNF agents compared with patients receiving DMARDs and healthy controls.

Methods

This study included 120 inflammatory arthritis patients receiving anti-TNF therapy and 116 patients on DMARDs in a large ($n = 1668$), prospective, rheumatic disease cohort conducted at a single site in São Paulo, Brazil (Rheumatology Division, Hospital das Clínicas da Universidade de São Paulo), between March 2010 and April 2010, described in detail elsewhere [7]. The study was approved by the local Institutional Review Board (Comissão de Pesquisa e Ética do Hospital das Clinicas da Faculdade de Medicina da Universidade de São Paulo), and all participants signed the informed consent. The trial was registered at clinicaltrials.gov under NCT01151644.

Patients

All patients fulfilled their respective disease classification criteria for RA [12], AS [13] or PsA [14]. Patients were initially invited by letter to participate in the public health influenza A H1N1/2009 vaccine campaign at the immunization centre of our hospital. Blood samples were obtained from each participant immediately before and 21 days after vaccination.

The anti-TNF group included 41 RA and 79 SpA patients (57 AS and 22 PsA). The anti-TNF agents and dosage at vaccination were as follows: 54 infliximab (3–5 mg/kg body weight at 2 and 6 weeks and thereafter as recommended, every 6–8 weeks), 40 adalimumab (40 mg every other week) and 26 etanercept (50 mg/week). In addition, 116 inflammatory arthritis (41 RA, 75 SpA, 53 AS and 22 PsA) patients on traditional DMARD therapy (MTX, LEF, chloroquine or SSZ) with similar disease distribution ($P > 0.05$) were randomly selected from the 462 inflammatory arthritis group patients of the large study [7].

Exclusion criteria were: previous known infection with pandemic 2009 influenza A H1N1, anaphylactic response to vaccine components or to eggs, acute infection resulting in a fever $>38^{\circ}$C at the time of vaccination, history of Guillain–Barré syndrome or other demyelination syndromes, previous vaccination with any live vaccine 4 weeks before the study or any inactivated vaccine 2 weeks before the study, previous vaccination with a 2010 seasonal influenza vaccine, a blood transfusion within the past 6 months, less than 8 weeks of anti-TNF therapy, hospitalization or failure to complete the protocol.

Healthy controls

One hundred and seventeen healthy subjects who came to this centre seeking vaccination in response to a Public Health National Campaign were invited to participate under the same exclusion criteria; these subjects were randomly selected from 234 healthy controls from the large study [7].

Vaccine

The H1N1 vaccine, a novel, monovalent, non-adjuvanted, inactivated, split-virus vaccine was produced by Butantan Institute/Sanofi Pasteur (São Paulo, Brazil). The active substance is a split, inactivated influenza virus containing antigens equivalent to the A/California/7/2009 (H1N1) virus-like strain (NYMCx-179 A), one of the candidate reassortant vaccine viruses recommended by the World Health Organization. The vaccine was prepared in embryonated chicken eggs with the same standard techniques that are used for the production of seasonal trivalent inactivated vaccines, and it was presented in 5-ml multi-dose vials, with thimerosal added as a preservative (45 μg/0.5 ml dose).

Study procedures

All subjects were vaccinated with the pandemic 2009 influenza vaccine (A/California/7/2009/Butantan Institute/Sanofi Pasteur). A single i.m. dose (0.5 ml) of 15-μg haemagglutinin antigen, specific for the H1N1 A/California/7/2009-like virus, was administered [7, 8].

Safety assessments

A 21-day diary card was given to each participant at entry with 13 (Yes or No) established reactions. This card included local reactions (pain, redness, swelling and itching) and systemic adverse events, such as arthralgia, fever, headache, myalgia, sore throat, cough, diarrhoea, rhinorrhoea and nasal congestion. Participants were required to return their diary cards at the end of the follow-up period (21 days after vaccination). All local reactions were considered to be related to the H1N1 vaccine. Recorded symptoms were checked by the investigators to determine the causality of solicited systemic adverse events, and unsolicited adverse events were also assessed. Severe side effects were defined as those requiring hospitalization or leading to death.

Laboratory assays

Blood samples were collected at baseline and 3 weeks after vaccination, and sera were stored at −70°C. The two samples from each patient or control were tested in parallel in the same plate for all laboratory determinations. The immunogenicity of the H1N1 A/California/7/2009-like virus vaccine was evaluated with the use of a haemagglutination inhibition assay (HIA) at the Adolfo Lutz Institute.

HIA

The influenza virus antigen used in this study was the H1N1 A/California/7/2009, supplied by the Butantan Institute. Virus concentrations were determined by haemagglutinin antigen titration, and the HIA test was performed after removing naturally occurring, non-specific inhibitors from the sera, as previously described [15]. The H1N1 vaccination immune response was evaluated by determining the levels of antibodies by HIA. Anti-H1N1 titre was determined by influenza HIA. The percentages of seroprotection (SP) (titre $\geqslant$1:40) and SC (pre-vaccination titre $<$1:10 and a post-vaccination HIA titre $\geqslant$1:40 or pre-vaccination titre $\geqslant$1:10 and a $\geqslant$4-fold increase post-vaccination), geometric mean titre (GMT) and the factor increase in GMT were calculated.

Statistical analysis

Selection of inflammatory arthritis patients on DMARDs and healthy controls was randomly carried out using SPSS Statistics v 15.0 (SPSS Inc., Armonk, NY, USA). Two-sided 95% CIs were calculated assuming binomial distributions for dichotomous variables and a log-normal distribution for HIA titres. Every subgroup had its HIA GMT calculated before vaccination and 21 days after vaccination. The factor increase in GMT (i.e. the ratio of the titre after vaccination to the titre before vaccination) was also obtained and log-transformed. Categorical variables were compared by Fisher's exact test or the chi-squared test. Normally or non-normally distributed variables were compared using the *t*-test or Wilcoxon rank-sum test, respectively. When comparisons of continuous variables were performed among more than two groups, one-way analysis of variance (ANOVA) or Kruskal–Wallis ANOVA was used. Multiple logistic regression modelling was applied to analyse the interaction between demographic characteristics, pre-vaccination status, medications and SC. All tests were two-sided, with a 0.05 significance level.

Results

Demographic data and current treatment

Inflammatory arthritis patients on anti-TNF therapy and healthy controls were of similar current age (45.1 ± 11.8 *vs* 44.3 ± 12.4 years, $P = 0.61$), a finding also observed for the comparison between inflammatory arthritis patients on anti-TNF and those on DMARDs (45.1 ± 11.8 *vs* 46.5 ± 10.6 years, $P = 0.44$). The frequency of female gender was significantly lower in anti-TNF compared with controls (50% *vs* 68%, $P = 0.0004$) and similar to DMARDs (50% *vs* 55.7%, $P = 0.43$). Mean disease duration was significantly higher in anti-TNF *vs* DMARD patients (18.4 ± 10.1 *vs* 15.6 ± 10.4, $P = 0.02$) (Table 1).

As expected, the frequencies of MTX (35.8% *vs* 53.4%, $P = 0.007$) and SSZ (15% *vs* 39.7%, $P = 0.0001$) use were significantly lower in patients under anti-TNF therapy compared with the DMARD group. No differences were observed in the frequencies and current doses of the other DMARDs, NSAIDs and immunosuppressive drugs in both groups ($P > 0.05$; Table 1).

Immunization response pattern in RA

Analysis of the immune response in RA patients revealed that before immunization the SP rate and GMTs were comparable in RA patients receiving anti-TNF therapy, those receiving DMARDs and healthy controls ($P > 0.05$). After immunization, the GMTs were significantly lower in patients on DMARDs ($P = 0.011$) compared with controls. Those using MTX showed a significant reduction in GMT ($P = 0.006$), factor increase in GMT ($P = 0.047$) and SP ($P = 0.018$) compared with controls, whereas reduced SC did not reach statistical significance ($P = 0.066$; Table 2). No differences in any parameters were evidenced in patients on mAbs and etanercept compared with healthy controls or those on DMARDs ($P > 0.05$; Table 2).

Immunization response pattern in SpA

Analysis of the immune response in SpA patients before immunization revealed comparable SP rates and GMTs in patients receiving anti-TNF therapy, those receiving DMARDs and healthy controls ($P > 0.05$). After immunization, SC ($P = 0.018$), SP ($P = 0.03$), GMT ($P = 0.005$) and factor increase in GMT ($P = 0.001$) were significantly lower in patients receiving anti-TNF therapy compared with healthy controls. The comparison of SpA patients receiving anti-TNF with those receiving DMARDs also

TABLE 1 Demographic data, disease distribution and treatment in patients on anti-TNF therapy, patients on DMARDs and healthy controls before pandemic 2009 influenza A H1N1 vaccination

Variable	Anti-TNF ($n = 120$)	DMARDs ($n = 116$)	Healthy controls ($n = 117$)
Demographic data			
Female gender	60 (50)*	67 (55.7)**	79 (68)
Current age, years	45.1 ± 11.8	46.5 ± 10.6	44.3 ± 12.4
Disease duration, years	18.4 ± 10.1***	15.6 ± 10.4	–
Diseases			
RA	41 (34.2)	41 (35.3)	–
SpA	79 (63.8)	75 (64.7)	
AS	57 (47.5)	53 (45.7)	–
PsA	22 (18.3)	22 (19.0)	–
Treatment			
Anti-TNF			
mAbs	94 (78.3)	–	–
Infliximab	54 (45.0)	–	–
Adalimumab	40 (33.3)	–	–
Soluble receptor			
Etanercept	26 (21.7)	–	–
Glucocorticosteroid	49 (40.8)	45 (38.8)	–
Current dose, mg/day	7.3 ± 3.2	9.6 ± 5.4	–
DMARDs			
MTX	43 (35.8)***	62 (53.4)	–
Current dose, mg/week	18.4 ± 6.3	19.2 ± 5.1	–
SSZ	18 (15.0)***	46 (39.7)	–
LEF	16 (13.3)	18 (15.5)	–
Chloroquine	11 (9.2)	18 (15.5)	–
Other drugs			
AZA	3 (2.5)	5 (4.3)	–
Ciclosporin	1 (0.8)	2 (1.7)	–
MMF	0	2 (1.7)	–
NSAID	36 (30.1)	41 (35.3)	–

Data are expressed as *n* (%) or mean (S.D.). *$P < 0.05$ (anti-TNF compared with age-matched randomly selected healthy controls), **$P < 0.05$ (DMARDs compared with randomly selected healthy controls), ***$P < 0.05$ (anti-TNF compared with randomly selected patients on traditional DMARDs).

revealed reduced SC ($P = 0.031$), GMT ($P = 0.024$) and factor increase in GMT ($P < 0.001$) in the former group. In addition, SP was also reduced but did not reach statistical significance ($P = 0.053$; Table 3). After immunization, the SC ($P = 0.002$), SP ($P = 0.006$), GMT ($P = 0.002$) and factor increase in GMT ($P < 0.001$) were significantly lower in SpA patients on mAb therapies (adalimumab or infliximab) compared with healthy controls. These same parameters were also significantly lower compared with those of patients receiving DMARDs ($P = 0.005$; $P = 0.014$; $P = 0.009$; $P < 0.001$, respectively) (Table 3).

Demographic data, pre-vaccination parameters, diseases (AS and PsA) and treatment of non-seroconverted ($n = 52$) *vs* seroconverted ($n = 102$) patients are illustrated in Table 4. The mean current age was significantly higher in non-seroconverted SpA patients compared with those who seroconverted (45.0 ± 11.3 *vs* 41.5 ± 10.3 years, $P = 0.003$). The frequency of anti-TNF ($P = 0.031$) and mAbs ($P = 0.001$) was significantly higher in patients who did not seroconvert compared with those who seroconverted, whereas the frequency of MTX use was lower in patients who did not seroconvert compared with those who seroconverted ($P = 0.028$; Table 4).

Multivariate logistic regressions were performed, including variables with $P \leqslant 0.2$ [current age, pre-vaccination GMT, MTX, LEF, disease (PsA or AS), mAbs and etanercept] and revealed that only older age ($P = 0.015$) and mAb treatment ($P = 0.023$) remained significant for non-SC.

Adverse events

Only mild systemic reactions were more often observed in patients on anti-TNF compared with healthy controls: fever (8.3% *vs* 0.9%, $P = 0.01$), arthralgia (12.5% *vs* 4.3%, $P = 0.03$) and nasal congestion (13.3% *vs* 4.3%, $P = 0.014$). No differences were observed in the frequency of adverse events in patients on anti-TNF compared with the DMARDs group ($P > 0.05$; Table 5). No severe adverse event was reported in any group after 3 weeks of follow-up.

Discussion

To our knowledge, this study was the largest analysis in inflammatory arthritis patients on distinct anti-TNF

TABLE 2 Serological data before and after pandemic 2009 influenza A H1N1 vaccine in RA patients and healthy controls

Variable	Pre-vaccination		Post-vaccination			
	GMT	SP	GMT	SP	FI	SC
Healthy controls ($n = 117$)	9.1 (7.8, 10.7)	11.1 (5.4, 16.8)	107.6 (83.6, 138.5)	78.6 (71.2, 86.1)	11.8 (9.3, 14.9)	74.3 (66.4, 82.3)
RA DMARD ($n = 41$)	6.8 (5.7, 8.1)	4.9 (1.7, 11.5)	56.1 (36.6, 86.0)*	63.4 (48.7, 78.2)	8.3 (5.4, 12.7)	61.9 (47.2, 76.6)
RA MTX ($n = 25$)	6.8 (5.5, 8.3)	0	43.5 (26.1, 72.5)*	56.0 (36.5, 75.5)*	6.4 (3.8, 10.8)*	56.0 (36.5, 75.5)
RA anti-TNF ($n = 41$)	7.4 (5.9, 9.2)	7.3 (0.6, 15.3)	66.4 (41.6, 106.1)	65.9 (51.3, 80.4)	9.0 (5.9, 13.7)	65.9 (51.3, 80.4)
mAbs ($n = 30$)	7.5 (5.7, 9.9)	6.7 (0, 15.6)	66.1 (36.1, 120.8)	66.7 (49.8, 83.5)	8.8 (5.1, 15.1)	66.7 (49.8, 83.5)
Etanercept ($n = 11$)	7.3 (5.1, 10.5)	9.1 (7.9, 26.1)	58.4 (30.1, 113.2)	63.6 (35.2, 92.1)	8.0 (4.6, 13.9)	63.6 (35.2, 92.1)

Data are expressed as percentage or value (95% CI). *$P < 0.05$ (RA DMARDs, RA MTX or RA anti-TNF compared with randomly selected healthy controls). FI: factor increase in GMT.

TABLE 3 Serological data before and after pandemic 2009 influenza A H1N1 vaccine in SpA patients and healthy controls

Variable	Pre-vaccination		Post-vaccination			
	GMT	SP	GMT	SP	FI	SC
Healthy controls ($n = 117$)	9.1 (7.8, 10.7)	11.1 (5.4, 16.8)	107.6 (83.6, 138.5)	78.6 (71.2, 86.1)	11.8 (9.3, 14.9)	74.3 (66.4, 82.3)
SpA DMARD ($n = 75$)	7.6 (6.4, 9.0)	6.7 (1.0, 12.4)	107.5 (74.3, 115.6)	78.7 (69.3, 88.0)	14.2 (10.1, 19.9)	74.7 (64.8, 84.6)
SpA MTX ($n = 35$)	8.2 (6.1, 11.1)	8.6 (0, 17.8)	176.7 (102.3, 305.1)	88.6 (78.0, 99.1)	21.5 (12.4, 37.4)	80.0 (66.7, 93.3)
SpA a-TNF ($n = 79$)	9.2 (7.5, 11.4)	11.4 (4.3, 18.4)	57.3 (41.5, 79.2)*,**,***	64.6 (53.9, 75.2)*,***	6.2 (4.6, 8.3)*,**,***	58.2 (47.3, 69.2)*,**,***
mAbs ($n = 64$)	9.0 (7.0, 11.5)	14.1 (5.5, 22.6)	50.2 (34.4, 73.4)*,**,***	59.4 (47.2, 71.5)*,**,***	5.6 (4.0, 7.8)*,**,***	51.6 (39.2, 63.9)*,**,***
Etanercept ($n = 15$)	10.5 (7.5, 14.7)	0	100.8 (64.1, 158.5)	86.7 (68.9, 100.0)	9.6 (6.9, 10.4)	86.7 (68.9, 100.0)

Data are expressed as percentage or value (95% CI). *$P < 0.05$ (SpA DMARDs or SpA anti-TNF compared with randomly selected healthy controls), **$P < 0.05$ (SpA anti-TNF compared with randomly selected SpA patients on DMARDs), ***$P < 0.05$ (SpA anti-TNF compared with randomly selected SpA patients on MTX). FI: factor increase in GMT.

classes, and clearly showed reduced immunogenicity in SpA patients on mAb therapies.

The major strength of this study was the inclusion of two randomly selected control groups. The absence of these control groups, specifically for the anti-TNF group, in the two previous studies evaluating pandemic influenza vaccine immune response precludes a definitive conclusion about the possible influence of other DMARDs [9, 10]. In addition, the separate evaluation of RA and SpA was an essential parameter to define more precisely the influence of a biologic agent on the immune response because a diverse pandemic vaccine immunogenicity profile in distinct autoimmune rheumatic diseases has been reported [7]. Moreover, the use of non-adjuvant vaccine was chosen to avoid autoimmune disease [16–18], although recent studies have reinforced the safety of adjuvanted influenza vaccine in rheumatic diseases [19]. On the other hand, the short observation period of the present study is a limitation and does not exclude long-term adverse events [20]. Furthermore, the influence of disease activity was not evaluated herein and must be clarified in future studies.

Biologic drugs may affect antibody production and vaccine immunogenicity [1, 2]. There are, however, controversial results regarding the humoral immune response after seasonal influenza immunization in patients with autoimmune rheumatic disease with either unaffected [21–23] or reduced immunogenicity [24–27].

Concerning the pandemic influenza vaccine, we have shown for the first time a distinctive immune response not only among RA and SpA patients but also between different anti-TNF agents. We have confirmed a previous observation that MTX [8, 9, 27] but not TNF blockage [8] therapy had a deleterious effect on influenza vaccination in RA patients.

The separate evaluation of the SpA group allowed for a more accurate definition of the effects of anti-TNF mAbs on the vaccine response in these diseases. In fact, mAbs seem to incur a higher risk for herpes zoster virus infection and tuberculosis than do soluble receptor TNF blockers [28, 29]. Additional studies are necessary to determine whether reported structural and functional differences among TNF blockers regarding pharmacokinetics, ability to cross-link transmembrane TNF, binding avidity and

TABLE 4 Comparison of pandemic 2009 influenza A H1N1 vaccine non-seroconverter SpA patients and seroconverters

Variable	Non-seroconverters ($n = 52$)	Seroconverters ($n = 102$)
Demographic data		
Female gender	17 (32.7)	31 (30.4)
Current age, years	45.0 ± 11.3*	41.5 ± 10.3
Disease duration, years	20.8 ± 12.6	16.7 ± 9.4
Pre-vaccination parameters		
SP	6 (11.5)	8 (7.8)
GMT	8.0 (95% CI 6.0, 10.6)	8.6 (95% CI 7.4, 10.0)
Diseases		
AS	35 (67.3)	75 (73.5)
PsA	17 (32.7)	27 (26.5)
Treatment		
Anti-TNF	33 (63.4)*	46 (49.7)
mAbs	31 (59.6)*	33 (32.4)
Etanercept	2 (3.8)	13 (12.7)
Glucocorticosteroid	8 (15.4)	15 (14.7)
Current dose, mg/day	9.1 ± 5.0	7.8 ± 4.1
DMARDs		
MTX	13 (25.0)*	44 (43.1)
Current dose, mg/week	16.3 ± 3.6	18.3 ± 6.5
SSZ	17 (32.7)	42 (42.2)
LEF	4 (7.7)	2 (2.0)

Data are expressed as *n* (%) and mean (S.D.). *$P < 0.05$ (non-seroconverters compared with seroconverters).

TABLE 5 Adverse events of pandemic 2009 influenza A vaccine in inflammatory arthritis patients on anti-TNF therapy, patients on DMARDs and healthy controls

Variable	Anti-TNF ($n = 120$)	DMARDs ($n = 116$)	Healthy controls ($n = 117$)
Local reactions	8 (6.7)	12 (10.3)	16 (13.7
Pain	6 (5.0)	6 (7.9)	14 (12.0)
Redness	0 (0)	0 (0)	4 (3.4)
Swelling	2 (1.7)	2 (1.8)	6 (5.1)
Itching	1 (0.8)	2 (1.8)	1 (0.9)
Systemic reactions	43 (35.8)	33 (28.4)	32 (27.4)
Fever	10 (8.3)*	4 (3.5)	1 (0.9)
Tremor	10 (8.3)	9 (7.9)	3 (2.6)
Arthralgia	15 (12.5)*	9 (7.9)	5 (4.3)
Headache	19 (15.8)	18 (15.8)	17 (14.5)
Myalgia	14 (11.7)	19 (16.7)	14 (12)
Diarrhoea	5 (4.2)	6 (5.7)	10 (8.5)
Sore throat	8 (6.7)	11 (9.6)	10 (8.5)
Cough	12 (10)	12 (10.5)	5 (4.3)
Rhinorrhoea	15 (12.5)	11 (9.6)	7 (6)
Nasal congestion	16 (13.3)*	12 (10.5)	5 (4.3)

Data are expressed as *n* (%). *$P < 0.05$ (anti-TNF compared with randomly selected healthy controls).

inhibition of cell activation and cytokine expression could ultimately affect vaccine antibody response [28, 30]. Moreover, the lower SC rate in patients treated with mAbs was not related to higher doses because only patients with the recommended standard dosage and interval for each TNF antagonist were included.

The uniformly low pre-vaccine SP in all groups, and absence in SpA patients under etanercept, may reflect the chance of acquiring a natural immunization, since the vaccine was not available in the previous year. However, post-vaccination immunogenicity in SpA patients on etanercept was adequate. The persistence of this antibody response for the next year needs to be evaluated in further studies.

Despite the similar ages in the three groups (anti-TNF, DMARDs and healthy controls), further analysis of non-seroconverting and seroconverting SpA patients confirmed on multivariate analysis that age influenced the pandemic influenza vaccination immune response [9, 31]. However, the small difference observed in the present study within a restricted age bracket may have no clinical relevance, despite the statistical significance.

Glucocorticoid therapy did not seem to influence immunogenicity in inflammatory arthritis patients, as also evidenced in RA and AS [10] and in SLE patients [32] who received the pandemic influenza vaccine. In contrast, current glucocorticoid [33] use was the major factor associated with decreased antibody production in a paediatric rheumatic disease population. Remarkably, the use of

DMARDs was not a predictive factor for a reduced humoral response in SpA, a pattern different from that observed in RA patients.

Of note, the influenza A (H1N1) vaccine was safe in inflammatory arthritis patients on anti-TNF therapies with predominantly mild systemic reactions. No serious short-term adverse event was observed, a finding reported previously in autoimmune rheumatic patients who received the seasonal influenza [21–25, 27] and pandemic vaccines [8, 9, 17–19, 32, 34, 35].

The European Committee for Medicinal Products for Human Use has suggested that all three criteria for vaccine immunogenicity should be met for pandemic vaccines [36]: SP >70%, SC >40% and factor increase in GMT >2.5 [37]. Despite a lower SC rate in patients receiving anti-TNF drugs, the majority achieved an adequate response, supporting the recommendation of this vaccine. Nevertheless, the second pandemic influenza A vaccination injection increased the immunogenicity of the rheumatic diseases [9, 17], supporting the notion that a booster may improve vaccine response in SpA patients on anti-TNF mAb therapy. In conclusion, this study revealed a distinct disease pattern of immune response in inflammatory arthritis patients receiving anti-TNF agents, with reduced immunogenicity solely in SpA patients using mAbs.

Rheumatology key messages

- Older age and anti-TNF mAbs reduced immunogenicity to pandemic 2009 influenza A H1N1 vaccine in SpA patients.
- Short-term safety after pandemic influenza vaccination was observed in inflammatory arthritis patients on anti-TNF treatment.

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Pandemic unadjuvanted influenza A (H1N1) vaccine in dermatomyositis and polymyositis: Immunogenicity independent of therapy and no harmful effect in disease☆

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ABSTRACT

The goal of the present study was to evaluate the influence of the influenza A H1N1/2009 vaccine on dermatomyositis/polymyositis (DM/PM) disease parameters and the potential deleterious effect of therapy on immune response. Thirty-seven DM and 21 PM patients (Bohan and Peter's criteria) were gender- and age-matched to 116 healthy controls. Seroprotection, seroconversion, the geometric mean titers (GMTs) and the factor increase (FI) in the GMTs were calculated. Disease safety was determined from a muscle enzyme analysis and the DM/PM scores [patient's visual analog scale (VAS), physician's VAS, manual muscle strength (MMT-8)] evaluated pre- and post-vaccination. The mean age (43.1 ± 9.9 *vs.* 43.8 ± 8.4 years, $p = 0.607$) and gender distribution ($p = 1.00$) were comparable between the patients and controls. After 21 days, seroconversion ($p = 0.394$), seroprotection ($p = 0.08$), GMT ($p = 0.573$) and the FI in the GMT ($p = 0.496$) were similar in both groups. The disease and muscle parameters remained stable throughout the study, including the creatine kinase ($p = 0.20$) and aldolase levels ($p = 0.98$), the physicians' VAS ($p = 1.00$), the patients' VAS ($p = 1.00$) and the MMT-8 ($p = 1.00$). Regarding the influence of treatment, the seroconversion rates were comparable between the controls and patients undergoing treatment with glucocorticoid (GC) ($p = 0.969$), GC >0.5 mg/kg/day ($p = 0.395$) and GC + immunosuppressors ($p = 0.285$). Vaccine-related adverse events were mild and similar in the DM/PM and control groups ($p > 0.05$). Our data support the administration of the pandemic influenza A H1N1/2009 vaccination in DM/PM, as we found no short-term harmful effects related to the disease itself and adequate immunogenicity in spite of therapy. Further studies are necessary to identify any long-term adverse effects in patients with these diseases.

1. Introduction

The pandemic influenza A (H1N1) emerged and rapidly spread worldwide in 2009, affecting mainly young population [1]. Its spectrum of clinical presentation varied from asymptomatic to respiratory failure and death [2]. Serious outcomes of influenza disease have been associated with risk factors such as underlying medical conditions, including obesity, pregnancy, cardiovascular diseases and immunosuppressive therapy [3]. Impairment of immune function inherent to the disease itself or secondary to drugs seems to underlie the higher risk in patients under immunosuppressant treatment [4], supporting the recent recommendations of the Advisory Committee on Immunization Practices and the European League Against Rheumatism that immunocompromised patients should receive the flu vaccine [5,6].

The efficacy and safety of vaccination with the monovalent pandemic adjuvanted H1N1 influenza were demonstrated by Elkayam et al. [7] and Gabay et al. [8] in a limited number of patients with rheumatic diseases. Previous seasonal vaccine studies did not include patients with idiopathic inflammatory myopathies, and evaluations of the safety of vaccines against other microbial agents for these patients are also scarce [9–13]. Another uncertain topic is the potential risk of flares of DM/PM following vaccination.

More recently, we reported the overall short-term safety, but reduced immunogenicity, of an adjuvant-free influenza A

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(H1N1) vaccine in a cohort of 1668 autoimmune rheumatic disease patients, including for the first time 73 dermatomyositis (DM)/polymyositis (PM) patients [14]. However, the vaccine's potential deleterious effects on the disease parameters and the possible influence of therapy on the vaccine antibody response have not been explored.

Therefore, the objectives of the present study were to evaluate the influence of influenza A H1N1/2009 vaccine in DM/PM disease parameters and the potential deleterious effect of therapy on the immunoresponse.

2. Materials and methods

2.1. Study design and participants

The present study was prospective and was conducted at a single center during the Public National Health pandemic 2009 influenza A (H1N1) vaccination campaign in Brazil. It was approved by the institutional review board of our university hospital and registered at ClinicalTrials.gov number: NCT01151644. The study included two stages: vaccination (March 22nd to April 2nd, 2010) and a follow-up period of 21 days with a personal diary card for reporting adverse events.

Out of 73 adult DM/PM patients (Bohan and Peter's criteria) [15] who received the vaccination and regularly attended at the Idiopathic Inflammatory Myopathies Outpatient Clinics, 58 adult patients (37 DM and 21 PM) had complete serology, clinical and therapeutic data and were included in the study. Out of 234 healthy vaccinated individuals recruited from the hospital's immunization center, 116 gender- and age-matched individuals were randomly selected as controls.

2.2. Exclusion criteria

The exclusion criteria were a previous known infection with pandemic 2009 influenza A H1N1; a history of anaphylactic response to vaccine components or to eggs; an acute infection resulting in a fever of over 38 °C at the time of vaccination; a history of Guillain–Barré syndrome or demyelination syndromes, cancer, or other associated autoimmune diseases; vaccination with any live vaccine within a period of 4 weeks prior to the study, any inactivated vaccine within a period of 2 weeks prior to the study, or the 2010 seasonal influenza vaccine; a blood transfusion within a period of 6 months prior to the study; less than 8 weeks of anti-TNF therapy, hospitalization during the study; or failure to complete the protocol [14].

2.3. Vaccine

The vaccine, Sanofi Pasteur 2009 influenza A (H1N1) was a novel monovalent adjuvant-free vaccine (A/California/7/2009/Butantan Institute/Sanofi Pasteur). The active component was a split inactivated influenza virus containing 15 μg hemagglutinin (HA) from an influenza A/California/07/2009 (H1N1) virus-like strain (NYMCx-179A) per 0.5 mL dose [9]. It was available as 5 mL multidose vials with thimerosal (45 μg/0.5 mL dose) added as preservative and was stored at 2–8 °C until used.

2.4. Study procedures

All subjects were vaccinated with a single intramuscular dose (deltoid muscle) of the H1N1 vaccine using a 22Gx1.¼ in. needle. The patient's visual analog score (VAS) and physician's VAS [16,17], the manual muscle testing score (MMT-8) [18,19], and the blood sample collection were assessed before and 21 days after the vaccination. Muscle enzymes were determined using an automated kinetic method [creatine kinase (normal range: 24–173 IU/L) and aldolase (normal range: 1.0–7.5 IU/L)].

2.5. Safety assessments

A 21-day symptom diary-card for prospective completion was given to each participant following vaccination and returned 21 days later. All new symptoms, recorded or not, in the diary were reviewed by the investigators, and the causal relationship with the vaccine was assessed. All patients answered one specific (yes or no) question about their perception of the vaccine's interference with their disease activity. Local reactions were defined as local pain, redness, swelling and itching, whereas systemic reactions included arthralgia, fever, headache, myalgia, sore throat, cough, diarrhea, rhinorrhea and nasal congestion.

2.6. Laboratory assays

The immunogenicity of the H1N1 A/California/7/2009-like virus vaccine was evaluated (hemagglutination inhibition assay-HIA) at Adolfo Lutz Institute [13]. The antibody titers were assessed at baseline and 21 days post-immunization. The following serologic endpoints were evaluated: the seroprotection rate, defined as the percentage of patients with a titer ≥1:40, and the seroconversion rate, defined as the percentage of patients with a ≥fourfold increase in vaccination titer if the pre-vaccination titer was ≥1:10 or a post-vaccination titer ≥1:40 if the pre-vaccination titer was <1:10.

2.7. Statistical analysis

Two-sided 95% confidence intervals (CI) were calculated assuming binomial distributions for dichotomous variables and a log-normal distribution for hemagglutination inhibition titers. For the categorical variables, the statistical summaries included the rates of seroconversion and seroprotection; these rates were compared using Fisher's exact test. For every subgroup, the hemagglutination inhibition geometric mean titers (GMTs) were calculated before vaccination and 21 days after vaccination. The factor increase in the GMTs (*i.e.*, the ratio of the titers after vaccination to the titers before vaccination) was also obtained. The factor increases and the GMTs were compared between DM/PM patients and controls using Student's two-sided *t*-test with the log-transformed titers. The Wilcoxon signed rank test was performed to analyze paired and non-parametric data.

3. Results

3.1. Demographic characteristics

The mean current age was comparable in the DM/PM patients and controls (43.1 ± 9.9 *vs.* 43.8 ± 8.4 years, $p = 0.607$), with a similar frequency (75.9%) of female gender in both groups ($p = 1.00$). The disease duration was 7.3 ± 6.3 years.

3.2. The influence of the vaccine on disease parameters

Table 1 illustrates the DM/PM parameters and treatment status before and after the influenza A H1N1/2009 vaccination. The pre- and post-vaccination disease and muscle parameters were comparable [patients' VAS ($p = 1.00$), physicians' VAS ($p = 1.00$), MMT-8 ($p = 1.00$), creatine kinase ($p = 0.19$) and aldolase ($p = 0.98$)].

Glucocorticoid and immunosuppressive treatments remained unchanged throughout the study ($p > 0.05$), as shown in Table 1. The frequencies of the use of immunosuppressive therapy were as follows: methotrexate (39.7%), azathioprine (32.8%), chloroquine diphosphate (15.5%), cyclosporine (13.8%), mycophenolate

Table 1
Dermatomyositis/polymyositis parameters and treatment before and after influenza A H1N1/2009 vaccine.

Variables (reference values)	Pre-vaccination ($n = 58$)	Post-vaccination ($n = 58$)	p
DM/PM parameters			
Patient's VAS (0–10)	0 [0–1]	0 [0–1]	1.000
Physician's VAS (0–10)	0 [0–1]	0 [0–1]	1.000
MMT-8 (0–80)	80 [80]	80 [80]	0.500
Creatine kinase, IU/L (24–173)	145.5 [121–186]	167.5 [98–321]	0.200
Aldolase, IU/L (1.0–7.5)	4.6 [3.6–5.5]	4.4 [3.4–7.7]	0.980
Treatment			
Prednisone			
Current use	32 (55.2)	32 (55.2)	1.000
Dose, mg/day	9.7 (2.5–60)	9.7 (2.5–60)	1.000
Prednisone ≥0.5 mg/kg/day	9 (15.5)	9 (15.5)	1.000
Methotrexate			
Current use	23 (39.7)	23 (39.7)	1.000
Dose, mg/week	8.5 (7.5–30)	8.5 (7.5–30)	1.000
Azathioprine			
Current use	19 (32.8)	19 (32.8)	1.000
Dose, mg/day	62.1 (100–300)	62.1 (100–300)	1.000
Cyclosporine			
Current use	8 (13.8)	8 (13.8)	1.000
Dose, mg/day	24.1 (100–300)	24.1 (100–300)	1.000
Mycophenolate mofetil			
Current use	2 (3.4)	2 (3.4)	1.000
Dose, g/day	0.1 (1.5–2.0)	0.1 (1.5–2.0)	1.000
Cyclophosphamide			
Current use	1 (1.7)	1 (1.7)	1.000
Dose (g/m^2 body surface)	1.2	1.2	1.000
Chloroquine diphosphate			
Current use	9 (15.5)	9 (15.5)	1.000
Dose, mg/day	250	250	1.000
Leflunomide			
Current use	2 (3.4)	2 (3.4)	1.000
Dose, mg/day	20	20	1.000
Immunosuppressive current use			
None	19 (32.8)	19 (32.8)	1.000
One immunosuppressive	23 (39.7)	23 (39.7)	1.000
Two immunosuppressive	16 (27.6)	16 (27.6)	1.000

Values are expressed in n (%) or median [interquartile range], median (range) or mean ± standard deviation (SD). DM, dermatomyositis; PM, polymyositis; MMT, manual muscle testing score.

mofetil (3.4%) and leflunomide (3.4%). Twenty-three (39.7%) out of 58 patients were using one immunosuppressive, six (27.6%) were receiving two immunosuppressives and 42 (74.1%) were simultaneously using immunosuppressive and glucocorticoid therapies. In addition, 32 (55.2%) patients were using prednisone with a mean dose at 9.7 mg/day, and nine (15.5%) of them were receiving ≥0.5 mg/kg/day.

3.3. Vaccine immunogenicity in DM/PM patients

The seroconversion rate ($p = 0.394$), the seroprotection rate (pre- and post-vaccination: $p = 0.234$ and $p = 0.08$, respectively), the GMTs ($p = 0.932$ and $p = 0.573$) and the factor increases in the GMTs ($p = 0.496$) were similar in the DM/PM patients and control groups (Table 2).

3.4. The influence of treatment on the vaccine immune response

The analysis of the patients' therapies revealed that DM/PM patients receiving glucocorticoid treatment had similar seroconversion rates ($p = 0.969$), seroprotection rates (pre- and post-vaccination: $p = 0.273$ and $p = 0.27$, respectively), GMTs ($p = 0.952$ and $p = 0.435$) and factor increases in the GMTs ($p = 0.403$) to the control group. Likewise, patients receiving a high dose of glucocorticoid treatment (≥0.5 mg/kg/day) had seroconversion rates ($p = 0.395$), seroprotection rates (pre- and post-vaccination: $p = 0.209$ and $p = 0.667$, respectively), GMTs ($p = 0.446$ and $p = 0.292$) and factor increases in the GMTs ($p = 0.501$) comparable to those of controls. The concomitant use of glucocorticoid and immunosuppressive therapy also resulted in a comparable immune response to controls [seroconversion rate ($p = 0.285$), seroprotection rate (pre- and post-vaccination: $p = 0.553$ and $p = 0.066$, respectively), GMTs ($p = 0.786$ and $p = 0.846$) and factor increases in the GMTs ($p = 0.714$)], Table 2.

Moreover, the disease parameters (patients' VAS, physicians' VAS, MMT-8, creatine-kinase and aldolase) were comparable between seroconverted and non-seroconverted patients ($p > 0.05$) (data not shown).

3.5. Adverse events

The vaccine was well tolerated without any severe adverse effects during the follow-up. The frequencies of minor local reactions (8.6 *vs.* 11.2%, $p = 0.597$) and of mild systemic reactions (15.5 *vs.* 25.7%, $p = 0.123$) were similar between the patients and controls.

4. Discussion

To our knowledge, this is the largest study addressing short-term disease safety of adjuvant-free 2009 influenza A (H1N1) vaccine in patients with DM/PM. The vaccine did not seem to have a deleterious effect on disease and immunoresponse was not affected by therapy.

The advantages of the present study were the prospective design and the inclusion of well-defined DM/PM [14] patients with the careful exclusion of patients with cancer and other associated-autoimmune diseases. In addition, the patients were age- and gender-matched with the control group because

Table 2
Serological data before and after influenza H1N1/2009 vaccine in controls and dermatomyositis/polymyositis patients.

Subset	Pre-vaccination		Post-vaccination		Factor increase	Seroconversion
	GMT	Seroprotection	GMT	Seroprotection		
Controls (n = 116)	8.9 (7.7–10.3)	10.3 (4.8–15.9)	102.8 (82.8–127.8)	84.5 (77.9–91.1)	11.6 (9.3–14.4)	78.4 (70.9–86.0)
DM/PM (n = 58)	8.8 (6.9–11.1)	17.2 (7.4–27.0)	119.0 (75.3–188.1)	72.4 (60.8–84.0)	13.6 (9.1–20.3)	72.4 (60.8–84.0)
Using GC (n = 32)	9.0 (7.0–11.5)	18.8 (8.5–29.0)	135.3 (83.7–218.7)	75.0 (63.7–86.3)	15.1 (9.9–23.0)	78.1 (67.3–88.9)
Using GC >0.5 mg/kg/day (n = 9)	12.6 (9.0–17.6)	33.3 (20.5–46.2)	201.6 (127.9–317.7)	77.8 (66.4–89.1)	16.0 (11.3–22.6)	88.9 (80.3–97.5)
Using IS and GC (n = 43)	8.5 (6.8–10.7)	14.0 (4.9–23.0)	109.1 (67.7–175.9)	69.8 (57.8–81.7)	12.8 (8.4–19.6)	69.8 (57.8–81.7)

Data are expressed in percentages or value (95% CI). DM, dermatomyositis; factor increase, in GMT pos-vaccination; GC, glucocorticoid; GMT, geometric mean titer; IS, immunosuppressive; PM, polymyositis.
All subsets *vs.* with control group showed $p > 0.05$.

immunogenicity varies according to age [13] and gender [20]. We also choose the non-adjuvanted preparation to minimize the potential risk of flares of underlying autoimmune diseases and the risk of "adjuvant disease" in genetically susceptible individuals [21] is defined as autoimmune syndrome induced by adjuvants (ASIA syndrome) [22,23].

We have established the disease safety of the pandemic H1N1 influenza vaccine on the basis of the result that muscle enzymes and DM/PM scores remained stable throughout the study; muscle enzymes and DM/PM scores are well-known indicators of myositis activity in the clinical management of these idiopathic inflammatory myopathies [16–19].

Additionally, we have confirmed our previous finding that the seroconversion of the H1N1 pandemic vaccine is adequate and comparable in age-matched healthy controls in contrast to the reduced vaccine response in patients with juvenile autoimmune rheumatic diseases [24], systemic lupus erythematosus [25] and rheumatoid arthritis [7,26]. One possible explanation for this discrepancy is the fact that the majority of the patients were stable with respect to laboratory and clinical parameters. Notably, the seroconversion rate was also not affected by glucocorticoid and immunosuppressive therapies whereas a deleterious effect of these drugs was reported in patients with systemic lupus erythematosus [8], rheumatoid arthritis [7,26], ankylosing spondylitis [7] and pediatric rheumatic diseases [24].

We further demonstrated that post-vaccination seroprotection in DM/PM patients was in fact similar to that in controls when compared to a rigorously gender- and age-matched group. The non-attendance for each subgroup analyzed in our previous study evaluating a large cohort of rheumatic disease patients may explain the reduced response reported for DM patients [14].

No serious short-term adverse events were observed, as reported previously in patients with autoimmune rheumatic disease who received seasonal influenza [7] and pandemic vaccines [8,24–26]. Long term effects on DM/PM could not be ruled out on the basis of the data presented because of the limited observation period of this study. This result should be interpreted with caution because the overall number of DM/PM patients was relatively small to detect relatively infrequent adverse events.

In summary, our data support the administration of the pandemic unadjuvanted influenza A H1N1 2009 vaccine in DM/PM patients on the basis of our findings of no short-term harmful effects related to the disease itself and the adequate immunogenicity of the vaccine in spite of therapy.

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Short and long-term effects of pandemic unadjuvanted influenza A(H1N1)pdm09 vaccine on clinical manifestations and autoantibody profile in primary Sjögren's syndrome

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ABSTRACT

Despite WHO recommendations about the A/California/7/2009/H1N1-like virus vaccination, studies evaluating its possible influence on clinical manifestations and autoantibody profile in primary Sjögren's syndrome (SS) are scarce. The aim of this study was to evaluate the possible influence of the unadjuvanted A/California/7/2009/H1N1-like virus vaccination on clinical manifestations and autoantibody profile in SS in the short/long-term. Thirty-six SS patients (The American-European Consensus Group Criteria, 2002) and 36 healthy controls with comparable mean age and gender were evaluated before and 21-days after this vaccination regarding seroprotection/seroconversion, factor increase in geometric mean titer (FI-GMT) and side effects. New onset of disease flares and autoantibody profile [antinuclear antibodies, anti-dsDNA, anti-Ro(SSA)/La(SSB), anti-RNP/anti-Sm, rheumatoid factor, anti-alpha-fodrin, anticardiolipin and anti-beta2-glycoprotein-I] were assessed before, 21-days and 1-year after vaccination. Patients and controls had similar rates of seroconversion (77.8 vs. 69.4%, $p = 0.42$), seroprotection (83.3 vs. 72.2%, $p = 0.26$) and FI-GMT ($p = 0.85$). Disease duration, prednisone (2.1 ± 4.9 mg/day), methotrexate and azathioprine did not affect seroconversion ($p > 0.05$). Regarding short-term, no change in the frequency or levels of autoantibodies was observed ($p > 0.05$) and only mild side effects were reported in comparable rates to controls ($p > 0.05$). During 1-year follow-up, the frequency of new disease flares was similar to the previous year (11 vs. 19%, $p = 0.51$), and four patients developed positivity to one of the following specificities: anti-Ro(SSA)/anti-La/(SSB), anti-alpha-fodrin, or IgM anticardiolipin. None developed specific lupus autoantibodies. Of note, a significant increase in the mean levels of anti-Ro/SSA ($p = 0.0001$) and anti-La/SSB ($p = 0.002$) was detected after 1-year with no change in the other autoantibodies. This is the first study indicating that influenza A(H1N1)pdm09 vaccine induces long-term changes in autoantibody profile restricted to SS spectrum without a deleterious effect in disease course.

1. Introduction

Primary Sjögren's syndrome (SS) is a chronic autoimmune disease characterized primarily by involvement of the exocrine glands, leading to sicca syndrome. Multiple organs may also be affected, causing a highly variable spectrum of clinical manifestations. Most patients have less severe extraglandular symptoms such as fatigue, polyarthralgia and diffuse myalgia, while others develop serious systemic impairments such as pneumonitis, vasculitis, peripheral neuropathy, glomerulonephritis, optic neuritis, multiple sclerosis-like disease and even lymphoma [1]. The etiology is unknown, but the autoimmune nature of SS is supported by the production of multiple circulating autoantibodies [2]. Anti-Ro/SSA and anti-La/SSB are detected in up to 90% and 60% of SS patients, respectively [2], and they are included in the disease classification criteria [3].

As a consequence of multiple systemic affections, it is often necessary to use high doses of glucocorticoids, immunosuppressive drugs and biological agents for the treatment of SS patients [4]. Indeed, it is relevant that infections, particularly respiratory, are considered important causes of morbidity and mortality in

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this disease [5–7], and vaccination is the most effective preventive measure to control virus dissemination and to reduce associated complications [8]. Pandemic influenza occurs repeatedly, H1N1 (Spanish flu, 1918), A/H2N2 (Asian influenza, 1957), A/H3N2 (Hong Kong flu, 1968), H1N1 (Swine flu, 1976; Russian flu, 1977) strains and A/California/7/2009 [A(H1N1)pdm09] [9], and it is of particular concern for immunosuppressed patients, who may be very exposed to this infection. Indeed, immunocompromised patients have indications to receive vaccine for seasonal and pandemic influenza according to the European League Against Rheumatism [10] and the 2010 Recommendations of the Advisory Committee on Immunization Practices [11], but data regarding its immunogenicity and disease safety in SS are scarce. In fact, the only two available published reports including SS patients focused on an overall analysis of several autoimmune rheumatic diseases (ARD) and evaluated short-term ($n = 36$) [12] and six months ($n = 23$) [13] safety and immunogenicity of A(H1N1)pdm09 influenza [12] and seasonal and/or pandemic influenza vaccination [13]. Both studies showed adequate seroconversion rate and mild vaccine side effects in ARD patients [12,13], however without a particular evaluation of the SS group [12,13]. In this regard, a control group of healthy individuals with comparable mean ages and gender is an essential parameter [14] not fulfilled in the previous large cohort study of ARD particularly for SS patients [12]. In addition, the effect of vaccine on disease itself was not evaluated and this is a relevant issue for patients with autoimmune genetic background such as those with SS, since the adjuvanted and non-adjuvanted A/H1N1 vaccines in the general population were associated with the development of various immunological disorders such as Guillain–Barré syndrome, acute encephalomyelitis, thrombocytopenia and vasculitis [15].

In addition, there are no data on the possible influence of the unadjuvanted A/California/7/2009/H1N1-like virus immunization on the autoantibody profile in SS patients. In this regard, increased serum levels of anticardiolipin antibodies (aCL) were observed in patients with systemic lupus erythematosus (SLE) up to one year after seasonal influenza immunization [16]. Likewise, the seasonal and/or pandemic influenza vaccine induced the production of anti-extractable nuclear antigen (anti-ENA) antibodies in ARD patients; nevertheless a SS group was not analyzed [17]. Thus, the aim of the present study was to evaluate short/long-term effects of unadjuvanted influenza A/California/7/2009/H1N1-like virus vaccination on clinical manifestations and autoantibody profile in SS.

2. Patients and methods

For this study, all SS patients and healthy controls had been recruited during the Public Health Pandemic Influenza Vaccination Campaign between March and April 2010 in a large, prospective rheumatic disease cohort study conducted at a single center, Rheumatology Division, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo – HCFMUSP, São Paulo, Brazil, (described in detail elsewhere [12]). The three male SS patients of our cohort did not respond to this call.

The study was approved by the local institutional review board, and all participants signed the informed consent. The trial was registered at clinicaltrials.gov under NCT01151644.

2.1. Patients and healthy individuals

Thirty-six female SS patients (according to The American-European Consensus Group Criteria [3]) aged >18 years (mean age: 55.6 ± 14.7 years) with stable disease (without active neurological or renal affections, and without current vasculitis) were selected and regularly followed for one year at the out-patient Sjögren's Syndrome Clinic (Rheumatology Division, HCFMUSP). Patients with history of multiple sclerosis-like disease or Guillain–Barré syndrome were excluded. Thirty-six healthy females with comparable age (52.3 ± 11.3 years-old, $p = 0.29$) to the patients were included.

2.2. Vaccine

The A(H1N1)pdm09 vaccine, a novel, monovalent, unadjuvanted, inactivated and split-virus vaccine, was produced by Butantan Institute/Sanofi Pasteur (São Paulo, Brazil). The active substance is a split, inactivated influenza virus containing antigens equivalent to the A/California/7/2009 (H1N1) virus-like strain (NYMCx-179A), one of the candidate reassortant vaccine viruses recommended by the WHO. The vaccine was propagated in embryonated chicken eggs, with the same standard techniques that are used for the production of seasonal trivalent inactivated vaccines, and it was presented in 5-ml multidose vials, with thimerosal added as a preservative (15 μg/0.5 ml dose).

2.3. Study design

Participants were assessed immediately before and 21-days after vaccination to determine seroprotection and seroconversion by haemagglutination inhibition assay (HIA) (Adolfo Lutz Institute, São Paulo, Brazil). Side effects (local pain, fever, arthralgia and flu symptoms) were also evaluated through a diary card. Disease flares were evaluated in all patients retrospectively 1-year prior and prospectively 1-year after vaccination ($n = 36$). Analysis of autoantibody profile included antinuclear antibodies (ANA), anti-double-stranded DNA (anti-dsDNA), anti-Ro/SSA, anti-La/SSB, anti-α-fodrin, anti-U1RNP, anti-Sm, rheumatoid factor (RF), anticardiolipin (aCL) and anti-β2-glycoprotein-I (anti-β2GPI) antibodies, and it was performed in patients immediately before, 21-days ($n = 36$) and 1-year ($n = 20$) of vaccination. During the vaccination campaign, healthy volunteers were followed only in the short-term (21-days), thus analysis of autoantibodies 1 year after immunization was performed only for SS patients.

2.4. Vaccination

All subjects were vaccinated with the pandemic influenza vaccine (A/California/7/2009/H1N1-like virus), Butantan Institute/Sanofi Pasteur). A single intramuscular dose (0.5 ml) of 15 μg haemagglutinin antigen, specific for the A/California/7/2009 (H1N1)-like virus, was administered.

2.5. Safety assessments

A 21-day diary card was given to each participant at study entry with a list of 13 solicited adverse reactions. This written card included local reactions (pain, redness, swelling and itching) and systemic adverse events such as arthralgia, fever, headache, myalgia, sore throat, cough, diarrhea, rhinorrhea and nasal congestion. Participants were required to return their diary cards at the end of the follow-up period (21 days after vaccination). All local reactions were considered to be related to the A(H1N1)pdm09 vaccine. Severe side effects were defined as those requiring hospitalization or leading to death. In addition, all SS patients were also evaluated regarding new onset of disease flares (parotiditis, arthritis, vasculitis, pneumonitis or neurological disorders) retrospectively 1-year prior and prospectively 1-year after vaccination. Medical charts were extensively reviewed for additional clinical and treatment data.

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2.6. Laboratory assays

Blood samples from patients and controls were collected at baseline and 3 weeks after vaccination for evaluation of the A(H1N1)pdm09 vaccination serological response. Sera were stored at −70 °C and the two samples from each patient or control were tested in parallel in the same plate. The immunogenicity of the A/California/7/2009 (H1N1)-like virus vaccine was evaluated with the use of a haemagglutination inhibition assay (HIA) at the Adolfo Lutz Institute. Samples were also collected from twenty patients 1 year after vaccination for evaluation of the profile of autoantibodies in the long term. The three samples from each patient (immediately before, 21-days and 1-year) of vaccination were tested in parallel in the same plate for all autoantibody determinations listed below.

2.7. Haemagglutination inhibition assay (HIA)

The influenza virus antigen used in this study was the A/California/7/2009 (H1N1) supplied by the Butantan Institute. Virus concentrations were determined by haemagglutinin antigen titration, and the HIA test was performed after removing naturally occurring, non-specific inhibitors from the sera, as previously described [18]. The A(H1N1)pdm09 vaccine immunoresponse was evaluated by determining levels of antibodies by haemagglutination inhibition. Anti-H1N1 titers were determined by influenza HIA. The percentages of seroprotection (SP) (titer ≥1:40) and seroconversion (SC) (a pre-vaccination titer <1:10 and a post-vaccination HIA titer ≥1:40 or pre-vaccination titers ≥1:10 and a ≥4-fold rise post-vaccination), geometric mean titers (GMTs) and factor increases (FIs) in GMTs were calculated [12].

2.8. Autoantibodies

For the evaluations of several autoantibodies, three samples from each patient (immediately before, 21-days and 1-year after vaccination) were tested in the same assay and applied in duplicates. Antinuclear and anti-dsDNA antibodies were detected by indirect immunofluorescence using as substrates HEp-2 cells or *Crithidia luciliae*, respectively (INOVA Diagnostics Inc., San Diego, USA) [19]. Serum levels of anti-Ro/SSA, anti-La/SSB, anti-U1RNP and anti-Sm antibodies were determined by ELISA with the purified antigens according to manufacturer's instructions (INOVA Diagnostics Inc., San Diego, USA) [20]. Concentrations of anti-alpha-fodrin antibodies (IgG and IgA) were also measured by ELISA with the purified protein (ORGENTEC Diagnostika GmbH, Mainz, Germany) [21]. Rheumatoid factor (IgM-RF) was measured by ELISA (INOVA Diagnostics Inc., San Diego, USA). Serum antiphospholipid antibodies were also evaluated. IgG and IgM anticardiolipin antibodies (aCL) were tested by ELISA as previously described (INOVA Diagnostics Inc., San Diego, USA) [22]. IgG and IgM anti-beta2-glycoprotein-I (anti-β2GPI) were measured by ELISA (ORGENTEC Diagnostika GmbH, Mainz, Germany) as previously outlined [23].

2.9. Statistical analysis

Comparison between two groups (SS patients vs. healthy controls and SS patients with A(H1N1)pdm09 seroconversion vs. SS patients without seroconversion) was conducted using Student's *t* test or Mann–Whitney U test for continuous variables and Chi-squared test or Fisher's exact test for categorical variables when applicable. For analysis comparing multiple data of the same group, one-way repeated measures analysis of variance (ANOVA) for continuous data and McNemar's test for categorical data were performed. Results are shown as a proportion, or mean ± standard deviation (SD). Only two-tailed tests were applied. *p*-values <0.05 were considered to be statistically significant.

Table 1
Immunogenicity and side effects of A(H1N1)pdm09 vaccine – 21 days in SS patients and controls.

	SS *n* = 36	Controls *n* = 36	*p*-value
Age, years [mean ± SD]	55.6 ± 14.7	52.3 ± 11.3	0.29
Female [*n* (%)]	36(100)	36(100)	–
Seroprotection [*n* (%)]			
Before vaccination	4(11.1)	3(8.3)	1.00
After vaccination	30(83.3)	26(72.2)	0.26
Seroconversion (%)	28(77.8)	25(69.4)	0.42
Side effects (%)			
Local pain	5(13.9)	4(11.1)	1.00
Fever	2(5.6)	0	0.49
Arthralgia	6(16.7)	4(11.1)	0.74
Headache	6(16.7)	5(13.9)	1.00
Rhinorrhea	3(8.3)	5(13.9)	0.71
Sore throat	3(8.3)	8(8.3)	1.00
Cough	7(19.4)	3(8.3)	0.31
Sputum	4(11.1)	3(8.3)	1.00

SS = primary Sjögren's syndrome; *n* = number of patients; SD = standard deviation.

3. Results

3.1. Short-term evaluation

3.1.1. Immunogenicity and safety of A(H1N1)pdm09 vaccine in patients and controls

All SS patients and controls were female, with comparable mean ages (55.6 ± 14.7 vs. 52.3 ± 11.3 years-old, $p = 0.29$). Pre-vaccination seroprotection rates (11.1 vs. 8.3%, $p = 1.00$), seroconversion (77.8 vs. 69.4%, $p = 0.42$), and seroprotection after immunization (83.3 vs. 72.2%, $p = 0.26$) were similar in patients and controls, respectively (Table 1). GMT before [8.9 (6.5–12.3) vs. 8.1 (6.2–10.5), $p = 0.96$] and after immunization [95.1 (60.4–149.8) vs. 89.8 (57.9–139.2), $p = 0.791$], and FI-GMT [10.7 (6.7–16.9) vs. 11.1 (7.1–17.3), $p = 0.85$] were also comparable in patients and controls, respectively. Regarding short-term (21-days period) side effects, only mild reactions related to the vaccine were observed in comparable rates in patients and healthy individuals ($p > 0.05$) (Table 1).

3.1.2. Influence of clinical features and therapy on A(H1N1)pdm09 vaccine seroconversion (SC)

In SS patients, seroconversion rate was not affected by disease duration ($p = 0.59$), use of glucocorticoid ($p = 0.16$), methotrexate ($p = 0.65$), antimalarial ($p = 0.68$), azathioprine ($p = 0.21$), or concomitant use of glucocorticoid and immunosuppressive drugs ($p = 0.31$) (Table 2).

3.1.3. Short-term A(H1N1)pdm09 vaccine effects on disease flares and autoantibody profile

None of the SS patients had disease flares and no patient had new positive tests or increased titers of ANA, anti-dsDNA, anti-U1RNP/Sm, anti-Ro(SSA)/La(SSB), RF, anti-α-fodrin, aCL or aβ2GP1 ($p > 0.05$).

3.2. Long-term A(H1N1)pdm09 vaccine effects on disease flares and autoantibody profile

3.2.1. Disease flares 1-year prior and 1-year after vaccination

The prospective long-term evaluation of SS patients revealed that 11% had new disease flares during the 1-year period, but the rate of new flares was similar to the previous year (19%, $p = 0.51$) (Table 3).

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Table 2
A(H1N1)pdm09 vaccine seroconversion (SC) and clinical and therapeutic features of SS patients.

	SS H1N1 SC+ ($n=28$)	SS H1N1 SC− ($n=8$)	p-value
Age, years [mean ± SD]	54.2 ± 13.2	60.5 ± 17.7	0.29
Disease duration, years [mean ± SD]	10.6 ± 5.4	9.4 ± 4.6	0.59
Treatment			
Prednisone [n (%)]	8 (28.6)	0	0.16
-dose, mg/day [mean ± SD]	2.1 ± 4.9	0	0.32
-range, mg/day	0–20	–	–
Antimalarial drugs [n (%)]	8 (28.6)	3 (37.5)	0.68
Immunosuppressive agents	11 (39.3)	3 (37.5)	1.00
Azathioprine [n (%)]	2 (7.1)	2 (25)	0.21
-dose, mg/day [mean ± SD]	6.3 ± 23.2	25 ± 46.3	0.44
-range, mg/day	0–100	0–100	–
Methotrexate [n (%)]	8 (28.6)	1 (12.5)	0.65
-dose, mg/week (mean ± SD)	4.1 ± 6.7	2.2 ± 5.8	0.57
-range, mg/week	0–20	0–17.5	–
Mycophenolate mofetil [n (%)]	1 (3.6)	0	1.00
Prednisone + immunosuppressive agents (%)	7 (25)	0	0.31
Without any of the above medications (%)	12 (42.9)	3 (37.5)	1.00

SS = primary Sjögren's syndrome; SC = seroconversion; n = number of patients; SD = standard deviation.

3.2.2. Autoantibody profile prior and 1-year after vaccination

In the long-term, 4/20 (20%) SS patients presented new positive tests: one patient became positive for anti-Ro/SSA, another for anti-La/SSB, one became positive for IgA anti-alpha-fodrin, and the last patient for IgM anticardiolipin (with levels >40 MPL). None developed lupus specific autoantibodies. Of note, comparing antibody levels at study inclusion, at 21-days and 1-year after vaccination, significant increases in the levels of anti-Ro/SSA (100.0 ± 40.8 vs. 100.9 ± 40.3 vs. 114.1 ± 40.1 U, respectively; $p = 0.0001$) and anti-La/SSB (60.8 ± 42.0 vs. 60.5 ± 41.9 vs. 72.9 ± 47.2, respectively; $p = 0.002$) were observed after 1 year, whereas no change was detected for other antibodies specificities investigated (Table 4).

4. Discussion

This is the first study to indicate that influenza A(H1N1)pdm09 vaccine induces long-term changes in autoantibody profile restricted to SS spectrum without a deleterious effect in disease course. The long-term evaluation was a relevant aspect of this study, since increasing levels of autoantibodies induced by vaccination was a late phenomenon. In fact, a three months evaluation of autoantibody profile in A(H1N1)pdm09 immunization has not observed increased antibody levels in SLE patients [24], as also demonstrated herein for the 21-days assessment. We did not detect the occurrence of serious vaccine short-term adverse events and demonstrated adequate seroconversion and seroprotection rates particularly in SS. Our finding is reinforced by the comparable mean ages and gender in the groups of SS patients and healthy individuals, an essential parameter [14] not fulfilled in our previous large cohort study of autoimmune rheumatic diseases for these particular group of patients [12]. Glucocorticoid did not have a deleterious effect in vaccine humoral response probably related to the use of low doses of this drug. Indeed, doses higher than 20 mg/day were associated with a diminished A(H1N1)pdm09 vaccine antibody production in rheumatoid arthritis (AR) and systemic lupus erythematosus (SLE) [25,26]. Unlike evaluations of AR and SLE patients [25–27], immunosuppressive agents did not reduce vaccine immunogenicity, but the small representation of patients under this therapy in the present study hampers the interpretation of this finding. Vaccine did not trigger disease's exacerbations, since the frequencies of parotiditis, arthritis, vasculitis, and pneumonitis at 1-year follow-up were similar to the previous year. Additionally, our data suggest long-term vaccine safety in an autoimmune rheumatic disease, with no cases of neurological disorders such as Guillain–Barré syndrome, which was described months after immunization mainly with adjuvanted pandemic influenza vaccine in the general population [15].

Table 3
Disease flares 1-year prior and 1-year after A(H1N1)pdm09 vaccination in SS patients.

	1-Year prior vaccination ($n=36$)	1-Year after vaccination ($n=36$)	p-value
Parotiditis [n (%)]	3 (8.3)	2 (5.6)	1.00
Arthritis [n (%)]	6 (16.7)	2 (5.6)	0.26
Cutaneous vasculitis [n (%)]	1 (2.8)	0	1.00
Pneumonitis [n (%)]	1 (2.8)	2 (5.6)	1.00
Neurological disorders [n (%)]	0	0	–

SS = primary Sjögren's syndrome; n = number of patients.

The main limitation of our study is the evaluation of a group of SS patients with peculiar features, i.e. with stable disease and using low doses of glucocorticoids and immunosuppressant drugs. In contrast, in systemic lupus erythematosus (SLE), there are published data indicating that the unadjuvanted A(H1N1)pdm09 vaccine is safe in patients with active disease and using high doses of prednisone and immunosuppressants, but with significantly impaired immunogenicity [26].

Another important concern regarding vaccines is that they can occasionally stimulate autoantibody production or even a recently defined syndrome known as autoimmune/inflammatory syndrome induced by adjuvants (ASIA) [28]. In this regard, a case of primary Sjögren's syndrome following adjuvant influenza A(H1N1)pdm09 vaccination was described [29]. Symptoms started seven days after the immunization, with development of polyarthralgia, dry mouth, dry eyes, positive antinuclear antibodies, positive anti-Ro/SSA antibody and the presence of lymphocytic infiltrates in the salivary glands [29]. Analysis of samples from labial tissues using polymerase chain reaction method (PCR) showed the absence of the influenza A(H1N1)pdm09 RNA, indicating that there was no direct viral presence in the salivary glands [29]. The authors hypothesize that vaccine antigens could trigger the immune system by different mechanisms such as molecular mimicry, epitope spreading and polyclonal activation, leading to the outbreak of Sjögren's syndrome manifestations and the appearance of antinuclear and anti-Ro/SSA antibodies [29]. In this regard, we chose to use solely the non-adjuvanted vaccine because SS patients have theoretically a genetic predisposition for the development of autoimmune phenomena [1]. Furthermore, according to a systematic review on vaccination in patients with autoimmune rheumatic diseases carried by European League Against Rheumatism (EULAR), the safety regarding adjuvants in these diseases remains to be defined [30].

Importantly, the changes in autoantibody profile observed herein were restricted to SS spectrum and mainly in the levels of anti-Ro(SSA)/La(SS-B), raising the hypothesis that this antibody production may be a consequence of molecular mimicry. In this aspect, it is interesting that it was observed molecular mimicry between the antigen Ro and the Epstein–Barr virus (EBV) protein EBNA-1 [31,32].

Increased serum levels of these antibodies observed herein were not, however, associated with disease flares, as shown by clinical evaluation. Indeed, titers of anti-Ro antibodies may fluctuate during the course of the illness in both SLE and SS patients, but these changes were not associated with disease flares, with the exception of some patients with skin vasculitis [33]. A similar phenomenon was observed in SLE patients six months after non-adjuvanted A(H1N1)pdm09 immunization, with significantly higher levels of

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Table 4
Autoantibody profile immediately before, 21-days and 1-year after A(H1N1)pdm09 vaccination in SS patients.

	Before $n=20$	21-Days after $n=20$	1-Year after $n=20$	p-value
Anti-Ro/SSA				
-n (%)	18 (90)	18 (90)	19 (95)	1.00
-Serum levels [U, mean ± SD]	100.0 ± 40.8	100.9 ± 40.3	114.1 ± 40.1	0.0001
Anti-La/SSB				
-n (%)	12 (60)	12 (60)	13 (65)	1.00
-Serum levels [U, mean ± SD]	60.8 ± 42.0	60.5 ± 41.9	72.9 ± 47.2	0.002
Anti-U1RNP				
-n (%)	6 (30)	6 (30)	5 (25)	1.00
-Serum levels [U, mean ± SD]	31.0 ± 25.2	31.1 ± 28.0	30.7 ± 31.5	0.91
Rheumatoid factor IgM				
-n (%)	12 (60)	12 (60)	12 (60)	–
-Serum levels [U, mean ± SD]	105.9 ± 129.3	102.1 ± 119.9	90.8 ± 97.8	0.20
Anti-α-fodrin IgG				
-n (%)	1 (5)	1 (5)	1 (5)	–
-Serum levels [U, mean ± SD]	6.8 ± 6.3	6.8 ± 5.6	6.7 ± 6.2	0.85
Anti-α-fodrin IgA				
-n (%)	1 (5)	1 (5)	2 (10)	1.00
-Serum levels [U, mean ± SD]	6.9 ± 2.7	6.9 ± 2.2	7.6 ± 6.9	0.67
aCL IgG				
-n (%)	0	0	0	–
-Serum levels [GPL, mean ± SD]	8.8 ± 5.0	8.3 ± 4.5	8.1 ± 4.1	0.48
aCL IgM				
-n (%)	0	0	1 (5)	1.00
-Serum levels [MPL, mean ± SD]	10.9 ± 8.5	11.3 ± 8.2	10.7 ± 10.7	0.90
aβ2GPI IgG				
-n (%)	0	0	0	–
-Serum levels [U, mean ± SD]	7.2 ± 2.5	7.1 ± 2.9	6.4 ± 1.9	0.06
aβ2GPI IgM				
-n (%)	1 (5)	1 (5)	1 (5)	–
-Serum levels [U, mean ± SD]	11.5 ± 22.3	13.6 ± 29.2	10.5 ± 15.5	0.62

SS = primary Sjögren's syndrome; n = number of patients; SD = standard deviation.
aCL = anticardiolipin antibody; aβ2GPI = anti-β2-glycoprotein-I antibody.
Cut-off values: anti-Ro/SSA and anti-La/SSB: >40 U; anti-U1RNP: >40 U; rheumatoid factor: >20 U; anti-alpha-fodrin: >10 U; anticardiolipin (>40 GPL or MPL); anti-β2GPI > 20 U.

anti-Sm compared to baseline, but without clinical consequences [25].

In conclusion, influenza A(H1N1)pdm09 vaccine stimulates long-term production of SS related autoantibodies without a relevant change in clinical course. The additional observation of an adequate seroconvertion and seroprotection rates supports its recommendation in this disease.

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CLINICS

CLINICAL SCIENCE

Immunogenicity of influenza H1N1 vaccination in mixed connective tissue disease: effect of disease and therapy

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OBJECTIVE: To assess the potential acute effects regarding the immunogenicity and safety of non-adjuvanted influenza A H1N1/2009 vaccine in patients with mixed connective tissue disease and healthy controls.

METHODS: Sixty-nine mixed connective tissue disease patients that were confirmed by Kasukawa's classification criteria and 69 age- and gender-matched controls participated in the study; the participants were vaccinated with the non-adjuvanted influenza A/California/7/2009 (H1N1) virus-like strain. The percentages of seroprotection, seroconversion, geometric mean titer and factor increase in the geometric mean titer were calculated. The patients were clinically evaluated, and blood samples were collected pre- and 21 days post-vaccination to evaluate C-reactive protein, muscle enzymes and autoantibodies. Anti-H1N1 titers were determined using an influenza hemagglutination inhibition assay. ClinicalTrials.gov: NCT01151644.

RESULTS: Before vaccination, no difference was observed regarding the seroprotection rates ($p = 1.0$) and geometric mean titer ($p = 0.83$) between the patients and controls. After vaccination, seroprotection (75.4% *vs.* 71%, $p = 0.7$), seroconversion (68.1% *vs.* 65.2%, $p = 1.00$) and factor increase in the geometric mean titer (10.0 *vs.* 8.0, $p = 0.40$) were similar in the two groups. Further evaluation of seroconversion in patients with and without current or previous history of muscle disease ($p = 0.20$), skin ulcers ($p = 0.48$), lupus-like cutaneous disease ($p = 0.74$), secondary Sjögren syndrome ($p = 0.78$), scleroderma-pattern in the nailfold capillaroscopy ($p = 1.0$), lymphopenia $\leq 1000/mm^3$ on two or more occasions ($p = 1.0$), hypergammaglobulinemia ≥ 1.6 g/d ($p = 0.60$), pulmonary hypertension ($p = 1.0$) and pulmonary fibrosis ($p = 0.80$) revealed comparable rates. Seroconversion rates were also similar in patients with and without immunosuppressants. Disease parameters, such as C-reactive protein ($p = 0.94$), aldolase ($p = 0.73$), creatine phosphokinase ($p = 0.40$) and ribonucleoprotein antibody levels ($p = 0.98$), remained largely unchanged pre and post-vaccination. No severe side effects were reported.

CONCLUSIONS: The non-adjuvanted influenza A/H1N1 vaccination immune response in mixed connective tissue disease patients is adequate and does not depend on the disease manifestations and therapy.

KEYWORDS: Mixed Connective Tissue Disease; Influenza A Virus; H1N1 Subtype; Influenza Vaccine.

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INTRODUCTION

In 2009, there was a worldwide influenza A H1N1/2009 virus pandemic that caused many deaths and hospitalizations and resulted in the WHO subsequently including this virus in the trivalent seasonal flu vaccine (1,2).

Patients with systemic autoimmune diseases are particularly susceptible to infections. This complication is an important cause of mortality and morbidity, reinforcing the importance of vaccination in this subgroup of patients. Immunosuppressed patients have been overrepresented among those who have experienced severe influenza A H1N1/2009 virus infections, demanding specific recommendations for the vaccination (3).

One dose of the non-adjuvant split-virion vaccine appears to be effective and safe for people without rheumatic

No potential conflict of interest was reported.

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diseases (4). Regarding mixed connective tissue disease (MCTD), there is only one study in the literature from our group focusing solely on the side effects of the vaccine and the overall immune response in a large cohort of patients with systemic autoimmune diseases (5). However, there are no data regarding the influence of disease and therapy on the immunogenicity of influenza H1N1 vaccination in MCTD. The possible effects of this vaccine on the clinical and laboratory parameters of this disease are also unknown.

The aims of this study were therefore to evaluate the possible influence of disease and therapy on the vaccination immune response and the potential effect of the vaccine on the clinical and laboratory MCTD parameters.

METHODS

Study design and participants

This prospective study enrolled patients with MCTD from the Outpatient Clinic of the Rheumatology Division, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, between March 22, 2010, and April 2, 2010, during the public health influenza A H1N1/2009 vaccine campaign for immunosuppressed patients. The protocol was approved by the Local Institutional Ethics Committee and registered in clinicaltrials.gov under #NCT01151644.

All of the patients with an MCTD diagnosis regularly followed at rheumatology outpatient clinics were invited by letter to participate in the public health influenza A H1N1/2009 vaccine campaign at the Immunization Center of the same hospital. Healthy subjects who came to this center in response to the public health national campaign were invited to participate as control group.

After vaccination, there was a follow-up period of 21 days, during which the participants completed a personal diary card of side effects. The patients were clinically evaluated, and blood samples were collected pre- and 21 days post-vaccination. This period was chosen to evaluate the humoral response to influenza vaccine and is in accordance with previous studies (4).

We included the patients with MCTD who accepted the invitation and attended two visits for clinical and safety assessments and laboratory assays. All of the patients fulfilled Kasukawa's classification criteria (6). The healthy subjects were matched by gender and age and were included as the control group.

The participants were all ≥18 years of age and signed informed consent forms. The exclusion criteria were as follows: previous known infection with influenza A (H1N1) in 2009; anaphylactic response to vaccine components or egg proteins; acute infection with fever over 38℃ at the time of vaccination; history of demyelinating syndromes or Guillain-Barré syndrome; previous vaccination with any live virus vaccine four weeks before inclusion or with any inactivated virus vaccine two weeks before the recruitment; seasonal flu vaccination in 2010; or blood transfusion within 6 months and hospitalization.

The doses of steroids and/or immunosuppressive agents remained the same throughout the evaluation.

Vaccine

The H1N1 vaccine (batch #1002027) was produced by Butantan Institute/Sanofi Pasteur (São Paulo, Brazil) using a novel monovalent, unadjuvanted, inactivated, split-virus vaccine. The active substance was an inactivated split influenza virus containing antigen equivalent to the A/California/7/2009(H1N1) virus-like strain (NYMCx-179A), one of the candidate reassortant vaccine viruses recommended by the WHO. The vaccine was prepared in embryonated chicken eggs using the same standard techniques used to produce seasonal, trivalent, inactivated vaccine and was presented in 5 ml multi-dose vials with thimerosal added as a preservative (45 μg per 0.5 ml dose).

All of the participants received a single intramuscular dose (0.5 ml) of 15 μg of hemagglutinin antigen specific for pandemic H1N1 A/California/7/2009-like virus (A/California/7/2009/Butantan Institute/Sanofi Pasteur) administered by a member of the nursing staff from the Immunization Center of Hospital das Clínicas, Medical School, University of São Paulo. The 69 patients with MCTD and 69 healthy subjects were vaccinated from March 22 to April 2, 2010.

Clinical assessments

The immune responses of the patients were analyzed according to the clinical features that they presented after their diagnosis with MCTD: muscle disease (muscle weakness associated with at least a two-fold elevation of creatine phosphokinase and/or aldolase in the absence of thyroid disease, infections or myopathy-inducing drugs); skin ulcers; SLE-like cutaneous disease (malar rash or photosensitivity); secondary Sjögren syndrome according to the American European Consensus Group (AECG) criteria (7); a scleroderma-pattern in nailfold capillaroscopy (presence of avascular areas or enlarged loops associated with at least one additional SD-parameter: microhemorrhages, reduced capillary density, enlarged loops and avascular areas); lymphopenia ≤1000 cells/mm^3 on two or more occasions (not induced by cytotoxic drugs); hypergammaglobulinemia ≥1.6 mg/dl; pulmonary arterial hypertension (estimated systolic pulmonary arterial pressure ≥40 mmHg or estimated mean pulmonary arterial pressure >25 mmHg at echocardiogram); or pulmonary disease (presence of ground-glass opacity predominantly in the subcortical region at lower pulmonary lobes on chest high resolution computerized tomography).

Safety assessments

A 21-day diary card was given to each participant at the beginning of the study. This card listed 13 established side effects and requested yes or no responses. It also included the following items: local reactions (pain, redness, swelling and itching) and systemic adverse events (arthralgia, fever, headache, myalgia, sore throat, cough, diarrhea, rhinorrhea and nasal congestion). The diary cards were not pre-tested and were based on the adverse events reported in previous studies for the same vaccine in healthy subjects (4). The patients were instructed to return the cards 21 days after the vaccination for follow-up. All of the local reactions were considered to be related to the vaccine. The recorded systemic symptoms were checked by the investigators to determine the causality of solicited adverse events. Unsolicited adverse events were also assessed. Severe side effects were defined as those that required hospitalization or caused death.

Laboratory assays

The immunogenicity of the H1N1 A/California/7/2009-like virus vaccine was evaluated using a hemagglutination inhibition assay (HIA) at the Adolfo Lutz Institute (São Paulo, Brazil). The influenza virus antigen used in this study was the H1N1 A/California/7/2009 provided by the Butantan Institute (São Paulo, Brazil). The viral concentration was previously determined using hemagglutinin antigen titration, and the HIA test was performed after removing the naturally occurring non-specific inhibitors from the sera as previously described. The immune response to the H1N1 vaccination was evaluated by determining the level of antibodies using HIA. The anti-H1N1 titer was determined by influenza HIA. The percentage of seroprotection (titer ≥1:40), seroconversion (pre-vaccination titer <1:10 and post-vaccination HIA titer ≥1:40 or pre-vaccination titer ≥1:10 and post-vaccination titer ≥4-fold increase), geometric mean titers (GMTs) and factor increase in GMTs were calculated. The GMT is the geometric mean of the titers, the simple arithmetic mean of the logarithms of the last positive dilution of each serum. The factor increase in GMTs is the ratio of the GMT after vaccination to the GMT before vaccination.

The laboratory inflammatory activity of MCTD was evaluated pre- and post-vaccination by measuring the levels of aldolase, C-reactive protein (CRP) and creatine kinase (CK) using standard methods. The anti-ribonucleoprotein (anti-RNP) levels were also determined using an enzyme-linked immunosorbent assay (ELISA) with a commercially available kit (INOVA Diagnostics).

Statistical analysis

Two-sided 95% CIs were calculated assuming binomial distributions for the dichotomous variables (Clopper-Pearson method) and log normal distributions for the hemagglutination inhibition titers. The categorical variables were compared using Fisher's exact test; the normally or non-normally distributed variables were compared using a t-test or Mann-Whitney rank sum test. All of the tests were two-sided, with a 0.05 significance level.

RESULTS

Seventy-five patients with MCTD accepted the invitation to participate in the study, but only 69 returned for the clinical and safety assessments and laboratory assays and were included. Sixty-nine healthy controls were also studied. As expected, the mean age (48.6±12.6 *vs.* 48±12, $p=0.7$) and gender ratios (a female gender predominance; 95.6 *vs.* 95.6%, $p=1.00$) of the patients and controls were alike. The mean disease duration was 12.9±8.9 years. The frequencies of MCTD manifestations were as follows: pulmonary fibrosis (52.2%), pulmonary arterial hypertension (20.3%), myositis (47.8%), lymphopenia (39.1%) and hypergammaglobulinemia (59.4%). At the beginning of the study, thirteen (18.84%) of the patients were not taking any drugs. Thirty-one (44.9%) of the patients were taking prednisone with a mean dose of 10.5±7.2 mg/day. Current use of immunosuppressive agents was observed in 45 (65.2%) of the patients as follows: azathioprine (42.2%), methotrexate (28.9%), leflunomide (15.6%) and others (13.3%) (Table 1).

Table 1 - Laboratory data before and after the vaccination and treatment in 69 MCTD patients.

	MCTD patients (n = 69)		
	Before vaccine	After vaccine	*p*-value
Laboratory data			
ANTI-RNP (UI/ml)	2391.3 (1210.6)	2446.1 (1182.1)	0.98
CRP (mg/dl)	9.3 (13.4)	9.6 (13.9)	0.94
Aldolase	4.2 (2.8)	4.5 (3.0)	0.73
CK	199.3 (231.8)	153.1(152.7)	0.40
Treatment			
Corticosteroids, n (%)	31 (44.9)		
mean dose, mg/day	10.5 (7.2)		
Chloroquine, n (%)	25 (36.2)		
Azathioprine, n (%)	19 (27.5)		
mean dose, mg/day	137.5 (42.2)		
Methotrexate, n (%)	13 (18.8)		
mean dose, mg/day	18.2 (5.9)		
Leflunomide, 20 mg/day, n (%)	7 (10.1)		

Data are expressed as numbers (%) or means (SD) unless otherwise specified. The medications that were prescribed to less than 10% of the patients were not included. CRP, C-reactive protein; CK, creatine kinase.

Influenza A H1N1/2009 vaccine immunogenicity *vs.* healthy controls

At study onset, the seroprotection rates ($p=1.0$) and GMT ($p=0.83$) were similar between the patients and controls. After vaccination, the seroprotection rate (75.4% *vs.* 71%, $p=0.70$), seroconversion rate (68.1% *vs.* 65.2%, $p=1.0$) and factor increase in GMT (10.0 *vs.* 8.0, $p=0.40$) remained similar in both groups (Table 2).

Effect of therapy in the influenza A H1N1/2009 vaccine immune response

The comparison of MCTD patients post-vaccination with and without therapy revealed comparable seroprotection ($p=1.0$), seroconversion ($p=1.0$) and FI GMT ($p=0.61$). Similarly, the seroconversion rates were alike in patients with and without the following therapies: glucocorticoids ($p=0.80$), chloroquine ($p=0.79$), azathioprine ($p=0.26$), methotrexate ($p=1.0$) and leflunomide ($p=0.68$). Patients with and without immunosuppressive agents also had a similar post-vaccination seroprotection rate (75.6%; 95% CI, 62.3-88.9% *vs.* 75%; 95% CI, 59-91%; $p=1.0$), FI GMT (13.5; 95% CI, 8.2-22.1 *vs.* 6.4; 95% CI, 4.3-9.5; $p=0.06$) and seroconversion rate (73.2%; 95% CI, 59.4-86.9 *vs.* 57.1; 95% CI, 38.5-76%; $p=0.2$).

Effect of disease in the influenza A H1N1/2009 vaccine immune response

Analysis of clinical parameters revealed comparable rates of seroconversion in MCTD patients with and without current or previous history of the following factors: muscle disease (75.7%; 95% CI, 60.9-90.6% *vs.* 58%; 95% CI, 42-74.7%; $p=0.2$), skin ulcers (80%; 95% CI, 53.9 - 106.1% *vs.* 64%; 95% CI, 52.1-76,7%; $p=0.48$), SLE-like cutaneous disease (72.7%; 95% CI, 45.1-100.3% *vs.* 66%; 95% CI, 53.2-77.8%; $p=0.74$), secondary Sjögren syndrome (63.2%; 95% CI 40.9-85.4% *vs.* 67%; 95% CI, 54.1-80.6%; $p=0.78$), nailfold capillaroscopy scleroderma-pattern (65.8%; 95% CI, 50.5-81.0 *vs.* 67%; 95% CI, 46-87.3%; $p=1.0$), lymphopenia ≤1000 cells/mm^3 on two or more occasions (66.7%; 95%

Table 2 - Seroprotection, seroconversion, geometric mean titers and factor increase in geometric mean titers before and after vaccination.

	Pre-vaccination		Post-vaccination			
Subset	GMT	Seroprotection	GMT	Seroprotection	FI in GMT	Seroconversion
MCTD	8.3 (6.8-10.3)	10.1 (3.0-17.2)	83.3 (59.0-117.6)	75.4 (65.2-85.6)	10.0 (7.0-14.2)	68.1 (57.1-79.1)
Controls	8.3 (6.9-9.9)	10.1 (2.9-17.3)	66.1 (49.6-88.1)	71 (60.2-81.8)	8.0 (6.0-10.6)	65.2 (53.9-76.5)
Glucocorticoid						
Yes	8.9 (6.2-12.8)	13.0 (1.0-25.0)	78.2 (42.9-142.4)	74.0 (59.0-90.0)	8.7 (4.8-15.9)	65.0 (47.0-82.0)
No	7.9 (6.2-9.8)	7.9 (-0.7-16.6)	87.6 (59.9-128.2)	76.3 (62.6-90.0)	11.1 (7.5-16.4)	68.4 (53.4-83.4)
Chloroquine						
Yes	7.8 (5.7-10.6)	8.0 (-3.0-19.0)	67.7 (41.4-110.7)	76.0 (59.0-93.0)	8.7 (5.0-14.8)	64.0 (45.0-83.0)
No	8.6 (6.6-11.3)	11.3 (1.8-20.8)	93.6 (59.5-147.2)	75.0 (62.0-87.9)	10.8 (6.9-16.8)	68.1 (54.2-82.1)
Azathioprine						
Yes	7.7 (5.4-10.9)	1.1 (-0.05-1.16)	119.5 (55.9-255.1)	79 (60.0-98.0)	15.4 (2.0-31.3)	79.0 (60.0-98.0)
No	8.6 (6.7-11.0)	1.1 (1.0-1.23)	72.6 (50.3-104.7)	74 (61.7-86.3)	8.4 (5.7-12.4)	62.0 (48.4-75.6)
Methotrexate						
Yes	6.5 (4.2-9.9)	8.0 (-7.0-23.0)	49.5 (27.0-90.5)	77 (53-101.0)	7.6 (3.8-14.9)	69.0 (43.0-95.0)
No	8.8 (7.0-11.1)	10.7 (2.5-18.8)	93.9 (63.7-138.6)	75 (63.5-86.4)	10.6 (7.1-15.7)	66.0 (53.5-78.6)
Leflunomide						
Yes	8.2 (3.8-17.7)	14.3 (-13.7-42.3)	119.5 (55.9-255.1)	79.0 (60.0-98.0)	15.4 (2.0-31.3)	79.0 (60.0-98.0)
No	8.3 (6.7-10.3)	9.6 (2.2-17.0)	73.9 (53.4-102.5)	75.8 (65.0-86.5)	8.8 (6.4-12.1)	67.7 (56.0-79.4)

Data are expressed in percentages or values (95% CI). GMT, geometric mean titer; FI in GMT, factor increase in GMT after vaccination; MCTD, mixed connective tissue disease.

CI 48.6-84.8% *vs.* 68%; 95% CI, 52.8-82.2%; $p=1.0$), hypergammaglobulinemia ≥1.6 g/d (63.4%; 95% CI, 48.5-78.3 *vs.* 71%; 95% CI, 52.3-89.4%; $p=0.60$), pulmonary arterial hypertension (64.3; 95% CI, 38.2 - 90.3% *vs.* 67%; 95% CI 54.8 - 79.8%; $p=1.0$) and pulmonary fibrosis (63.9%; 95% CI, 47.9 - 79.8 *vs.* 69.7%; 95% CI, 53.8 - 85.6%; $p=0.80$).

Vaccine safety

No severe side effects were reported. The frequencies of minor local reactions were similar between the patients and controls (13% *vs.* 29%, $p=0.11$). The systemic reaction that was most frequently reported by patients was myalgia (11.5%), but the reported level was not different relative to that of the controls ($p=1.0$).

■ DISCUSSION

This is the first study to determine that the immune response to influenza H1N1 vaccine in MCTD patients is adequate and independent of the clinical aspects of the disease and therapy.

Regarding other rheumatic diseases, we and others have previously demonstrated appropriate pandemic 2009 influenza A (H1N1) response and vaccine safety in patients with rheumatoid arthritis, ankylosing spondylitis, systemic sclerosis, psoriatic arthritis, Behçet's disease, primary antiphospholipid syndrome, dermatomyositis, primary Sjögren syndrome, Takayasu's arteritis, polymyositis, granulomatous polyangiitis and juvenile autoimmune rheumatic disease (juvenile systemic lupus erythematosus (SLE), juvenile idiopathic arthritis, juvenile dermatomyositis, juvenile scleroderma, and vasculitis) compared with healthy controls (5,8,9). In contrast, similar studies with SLE patients have demonstrated an impairment in the immune response to influenza vaccination evaluated by the assessment of autoantibodies (10-12). It is possible that these discrepancies could be related to variations in the diseases, variations in the vaccines and the usage of several medications.

No harmful effect of the disease was observed, which may be related to the use of unadjuvanted vaccine in the present study. Unadjuvanted vaccines offer the theoretical advantage of minimizing the risk of potentiating the humoral response and avoiding the autoimmune/inflammatory syndrome induced by adjuvants (ASIA) (13). However, a large meta-analysis revealed no difference in the incidence of adverse events of autoimmune origin between subjects who received influenza vaccinations with and without adjuvant (14).

In contrast with SLE, in which several activity indexes are available and widely used (15) and there are well-defined serological markers, such as anti-dsDNA and complement levels (16), there are no such tools for MCTD. In SLE, controversy remains regarding whether disease flare occurs after immunization with H1N1 vaccination (10,17) and whether there are changes in the levels of SLE-related auto antibodies (10,18). In this group of MCTD patients, the stability of the clinical, laboratory and treatment parameters throughout the study supports the notion that pandemic H1N1 is not harmful to this disease.

Conversely, the possible influence of disease manifestation in the post-vaccination immune response is a matter of concern, but none of the clinical or laboratory MCTD parameters evaluated were associated with a diminished humoral response. In contrast, the efficacy of the H1N1 pandemic vaccine was impaired in lupus patients with lymphopenia (19), and in HIV-infected individuals, a lower mean nadir CD4 cell count and longer duration of infection were associated with reduced seroconversion (20).

Despite the use of immunosuppressive drugs, MCTD patients did not present any impairment in their immune response. The influenza vaccine response appears to differ in individual rheumatic diseases (5). In this regard, our large cohort analysis of 555 lupus patients revealed that immunomodulators appear to be a more relevant factor for a reduced pandemic vaccine immune response than the disease itself (21). Similarly, we observed a methotrexate-impaired H1N1 vaccine-induced humoral response in RA patients (22). In children with systemic autoimmune diseases, glucocorticoid was identified as the only drug that decreased seroconversion in multivariate analysis (9). Immunosuppressive drugs, such as methotrexate, azathioprine, leflunomide, mycophenolate

mofetil, cyclosporine and glucocorticoid, were associated with lower antibody titers in patients with inflammatory rheumatic diseases (9,11,23).

The non-adjuvanted influenza A/H1N1 vaccination immune response in MCTD patients is appropriate and independent of their disease manifestations and therapies. In addition, the overall vaccine safety supports its recommendation.

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AUTHOR CONTRIBUTIONS

Miossi R contributed to the design, planning, interpretation of the data and statistical analysis, and writing. Fuller R contributed to the design, planning, interpretation of the data, and writing. Moraes JC, Ribeiro AC, Saad CG, Aikawa NE contributed to the execution and planning. Miraglia J and Ishida MA contributed to the execution (laboratory). Bonfá E contributed to the design and planning. Caleiro MT contributed to the design, planning, execution, and writing.

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Effective seroconversion and safety following the pandemic influenza vaccination (anti-H1N1) in patients with juvenile idiopathic arthritis

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Objectives: To assess the vaccine response in juvenile idiopathic arthritis (JIA) as an extension of previous observation of immunogenicity and safety of a non-adjuvanted influenza A H1N1/2009 vaccine in a large population of juvenile rheumatic diseases. Moreover, to assess the possible influence of demographic data, disease subtypes, disease activity, and treatment on immunogenicity and the potential deleterious effect of the vaccine in the disease itself, particularly in the number of arthritis and inflammatory markers.

Methods: A total of 95 patients with JIA and 91 healthy controls were evaluated before and 21 days after vaccination, and serology for anti-H1N1 was performed by haemagglutination inhibition assay (HIA). Patient and physician visual analogue scales (VAS), Childhood Health Assessment Questionnaire (CHAQ), number of active joints, acute phase reactants, and treatments were evaluated before and after vaccination. Adverse events were also reported.

Results: JIA patients and controls were comparable regarding mean current age (14.9 ± 3.2 vs. 14.6 ± 3.7 years, $p = 0.182$). After vaccination, the seroconversion rate was significantly lower in JIA patients compared to controls (83.2% vs. 95.6%, $p = 0.008$), particularly in the polyarticular subtype (80% vs. 95.6%, $p = 0.0098$). Of note, JIA subtypes, number of active joints, acute phase reactants, CHAQ, patient and physician VAS, and use of disease-modifying anti-rheumatic drugs (DMARDs)/immunosuppressive drugs were similar between seroconverted and non-seroconverted patients ($p > 0.05$). Regarding vaccine safety, no deterioration was observed in the number of active joints and acute phase reactants during the study period.

Conclusion: Influenza A H1N1/2009 vaccination in JIA induces a lower but effective protective antibody response probably independent of disease parameters and treatment with an adequate disease safety profile.

In 2009, an H1N1 influenza pandemic was established resulting in high rates of hospitalization (1–10%) (1) and mortality (2.6–7.6%) (2, 3), particularly among immunosuppressed patients. Indeed, infection is recognized as an important additional cause of increased morbidity of paediatric rheumatic diseases under treatment with disease-modifying anti-rheumatic drugs (DMARDs) and anti-tumour necrosis factor (TNF) agents (4, 5).

In this regard, vaccination is a well-known effective tool against a variety of infectious agents including influenza infection (6); in 2010, the Advisory Committee on Immunization Practices (ACIP) recommended influenza A H1N1/2009 immunization for high-risk groups, including juvenile idiopathic arthritis (JIA) patients (7). More recently, the European League Against Rheumatism (EULAR) task force reinforced the importance of vaccination in immunosuppressed paediatric rheumatology patients (8).

Additionally, for pandemic influenza vaccines to be licensed, children, adolescents, and adults must meet all three current immunology standards proposed by the European Medicines Agency (EMEA) and the Food and Drug Administration (FDA): seroprotection (SP) > 70%, seroconversion (SC) > 40%, and a factor increase (FI) in the geometric mean titre (GMT) > 2.5 (9–11).

There are few data in the literature regarding the H1N1 influenza vaccine in JIA patients and all of them are restricted to overall safety and vaccine response (12–15). Malleson et al (14) evaluated 34 children with chronic arthritis and 13 controls, and found adequate immune responses regardless of the use of glucocorticoids or immunosuppressive agents. A low but comparable immunoresponse to seasonal influenza vaccine was achieved by 49 rheumatic disease patients as well as by a control group with chronic illnesses (13). The small number of patients and healthy controls and the inclusion of infants, a group with a distinct pattern of vaccine

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immune response (13, 14), precludes a definitive conclusion about their findings.

We recently performed a prospective study regarding the non-adjuvanted influenza A H1N1/2009 vaccine in 237 juvenile autoimmune rheumatic diseases, including 93 JIA patients, and showed a reduced immune response especially associated with glucocorticoid therapy, with short-term vaccine safety results (15). However, the overall comparable ages among patients and controls in that study may not be extended to each disease group (15). In addition, the possible role of demographic characteristics, disease subtypes, and disease activity in antibody response to the pandemic H1N1 vaccine in JIA patients and the impact of the vaccine in disease safety, particularly related to the number of arthritis and acute phase reactants, were not assessed.

Therefore, the aims of this study were to analyse the influenza A H1N1/2009 vaccine response in patients and age-balanced healthy controls with further stratification of certain variables that could influence immunogenicity. The potential deleterious effect in disease activity parameters was also evaluated.

Methods

All 169 JIA patients followed at the Paediatric Rheumatology Unit of the Children's Institute and the Rheumatology Division, Clinics Hospital, Faculty of Medicine, University of São Paulo were invited by letter to participate in the public health influenza A H1N1/2009 vaccine campaign at the Immunization Centre of the same hospital. Ninety-five patients with JIA according to International League Against Rheumatism (ILAR) criteria (16) agreed to participate in the study and fulfilled the inclusion criteria. Ninety-one healthy volunteers who came to this centre seeking vaccination in response to the Public Health National Campaign were included as the control group. All participants were ≥ 9 and ≤ 21 years old. This study was approved by the local ethical committee of our University Hospital and informed consent was obtained from all participants or their legal guardians. The study was registered at clinicaltrials.gov under number NCT01151644.

Vaccine

Vaccination was contraindicated in the following conditions: (a) anaphylactic response to vaccine components or to egg, (b) acute infection resulting in fever with a temperature > 38°C at the time of vaccination, (c) history of Guillain–Barré or demyelination syndromes, (d) and live virus vaccination 4 weeks before or any inactivated vaccine 2 weeks before the study, according to Centers for Disease Control and Prevention (CDC) recommendations (6). Furthermore, exclusion criteria for patients and controls included: hospitalization, blood transfusion in the past 6 months, previous immunization against seasonal influenza 2008, and confirmed infection by influenza A H1N1/2009.

All JIA patients and healthy controls received one dose of a monovalent inactivated anti-influenza vaccine containing 15 μg of haemagglutinin antigen equivalent to the A/California/7/2009 (H1N1) virus-like strain (NYMC X-179A) without adjuvant, propagated in embryonated chicken eggs provided by the Butantan Institute. Virus concentrations were determined by the haemagglutination assay titration as described previously (17) and virus stocks were aliquoted and stored at −70°C until used. The vaccine was stored in 5-mL multi-dose vials using thimerosal (45 μg per 0.5-mL dose) as the preservative.

Immunogenicity assessment

All JIA patients and controls were evaluated on the day of vaccination and then 3 weeks later. Serology against the H1N1 A/California/7/2009-like virus was performed by haemagglutination inhibition assay (HIA) at the Adolfo Lutz Institute on the day of vaccination and then 21 days later.

Sera were tested for antibodies against the H1N1 A/California/7/2009 influenza strain supplied by the Butantan Institute at an initial dilution of 1:10 and a final dilution of 1:2560. For calculation purposes, a value of 1:5 was assigned for negative titres and a value of 1:2560 for titres > 1:2560. Samples were tested in duplicate and geometric mean values were used in the analysis. Virus concentrations were determined previously by haemagglutinin antigen titration, and the HIA test was performed after removing naturally occurring non-specific inhibitors from the sera as described previously (17).

Appropriate endpoints included: seroprotection (percentage of subjects with HIA neutralizing antibody titre $\geq$ 1:40), seroconversion (percentage of subjects with either a pre-vaccination HIA titre < 1:10 and a post vaccination HIA titre $\geq$ 1:40 or a pre-vaccination HIA titre $\geq$ 1:10 and a minimum fourfold rise in post-vaccination HI antibody titre); and an increase in geometric mean titre (GMT) (18).

Safety assessment

On the day of vaccination, all participants received a 21-day personal diary card containing the following list of predefined adverse events to be registered: local reactions (itching, pain, redness, and swelling at injection site) and systemic reactions (fever, malaise, chills, headache, arthralgia, myalgia, diarrhoea, cough, expectoration, sore throat, nasal congestion, and rhinorrhoea) (15). All local reactions were considered to be related to the influenza A H1N1/2009 vaccine, while systemic adverse events were analysed by the investigators to determine their causality. Severe side-effects were defined as those requiring hospitalization or death.

Clinical, laboratory, and therapy evaluations of JIA

Clinical and laboratory assessments of JIA patients were evaluated on the day of vaccination and after 3 weeks and included: number of active joints (swelling within a joint, or limitation in the range of joint movement with joint pain or tenderness), patient and physician global assessment of arthritis activity measured in mm on a 100-mm horizontal visual analogue scale (VAS) and the validated Brazilian version of the Childhood Health Assessment Questionnaire (CHAQ) (19). Erythrocyte sedimentation rate (ESR) was performed according to the Westergreen method and C-reactive protein (CRP) according to nephelometry. Current treatment with prednisone, DMARDs [methotrexate (MTX), leflunomide, and chloroquine], immunosuppressive drugs (cyclosporin), and anti-TNF agents (adalimumab, etanercept, and infliximab) was determined.

Statistical analysis

The difference between seroconversion rates in JIA patients and controls was calculated by Fisher's test with $\alpha = 0.05$. The size sample provided a power of 80% to find differences of at least 1/8 (12.7%) (Graphpad StatMate 1.01). The immunogenicity and safety analyses were descriptive, and the two-sided 95% confidence intervals (CIs) were calculated assuming binomial distributions for dichotomous variables and log-normal distribution for HIA titres. The GMTs were compared between JIA patients and the healthy controls using a two-sided Student's t-test or the Mann–Whitney U-test on the $\log_{10}$-transformed titres. Categorical variables (rates of seroprotection and seroconversion, prednisone and immunosuppressive drug use, and adverse events) were compared using Fisher's exact test. The effects on disease activity before and after vaccination were analysed with the Wilcoxon signed ranks test. The statistical significance was set at p-value < 0.05.

Results

JIA patients and controls were comparable regarding mean current age (14.9 ± 3.2 vs. 14.6 ± 3.7 years, $p = 0.182$) and female gender frequency (55.8% vs. 51.6%, $p = 0.659$). Mean disease duration was 7.6 ± 4.6 years. Regarding JIA subtypes, 45 (47.4%) were polyarticular, 24 (21%) oligoarticular, 18 (18.9%) systemic, and eight (8.4%) others. Sixty-three (66.3%) patients were taking at least one DMARD/immunosuppressive agent (prednisone, MTX, leflunomide, cyclosporin, sulfasalazine, anti-TNF agents, and/or abatacept) and 16 (16.8%) were under anti-TNF therapy.

Vaccine immunogenicity

Seroprotection and seroconversion rates, GMT, and FI in GMT in JIA patients and healthy controls are shown in Table 1. After 21 days, the seroconversion rate was significantly lower in JIA patients *versus* controls [83.2% (95% CI 75.6–90.7) vs. 95.6% (95% CI 91.4–99.8), $p = 0.008$]; however, both JIA patients and controls had adequate responses according to the EMEA/FDA standards, given that seroprotection was > 70%, seroconversion > 40%, and a GMT increase of > 2.5. The subanalysis of JIA subtypes showed that only polyarticular onset patients had statistically reduced seroconversion compared to controls [80% (95% CI 68.2–91.8) vs. 95.6% (95% CI 91.4–99.8), $p = 0.0098$], whereas no difference was found in oligoarticular ($p = 0.157$), systemic ($p = 0.087$), and enthesitis-related arthritis (ERA) ($p = 0.35$) patients. The 12 (26.7%) rheumatoid factor (RF)-positive polyarticular JIA patients had lower seroconversion rates ($p = 0.033$) compared to controls, as did the 33 RF-negative polyarticular JIA patients ($p = 0.022$) (Table 1). The use of immunosuppressive drugs in polyarticular JIA patients was significantly higher than in oligoarticular patients (80% vs. 41.7%, $p = 0.0027$) and similar to that in systemic patients (80% vs. 55.6%, $p = 0.063$). Regarding treatment influence, no difference was observed in immunogenicity parameters between patients with and without immunosuppressive drugs, as well as between subjects with and without MTX and TNF blockers (Table 1).

Influence of disease parameters and treatment in the vaccine response of JIA patients

Demographic data analysis revealed that female gender predominance ($p = 0.412$), mean current age ($p = 0.086$), and disease duration ($p = 0.449$) were comparable in seroconverted and non-serocoverted patients. The frequencies of JIA subtypes were similar in both groups ($p > 0.05$). The median of number of active joints, ESR, CRP, patients' VAS, physicians' VAS, and CHAQ were similar in seroconverted and non-serocoverted patients ($p > 0.05$). Regarding treatment, no difference was observed in the frequencies and doses of each therapy in both groups ($p > 0.05$) (Table 2). Furthermore, all disease parameters and treatments were similar between seroprotected and non-seroprotected JIA patients ($p > 0.05$), as well as between JIA patients who achieved FI in GMT > 2.5 and those who achieved FI in GMT ≤ 2.5 ($p > 0.05$).

Disease safety

The median number of active joints [0 (0–28) vs. 0 (0–18), $p = 0.552$)], CRP values [1.9 (0.1–137.3) vs. 2.7 (0.2–122.8) mg/dL, $p = 0.073$], and CHAQ score [0.123 (0–3) vs. 0 (0–3), $p = 0.058$] remained stable throughout the study. However, the medians for ESR [19 (1–83) vs. 15 (0–83) mm/1st hour, $p = 0.016$], patient VAS [10 (0–80) vs. 8.5 (0–80), $p = 0.001$], and physician VAS [10 (0–90) vs. 6 (0–80), $p = 0.002$] were statistically lower in the post-vaccination evaluation (Table 3).

AQ1 Table 1. Immunogenicity of influenza A H1N1/2009 vaccine in juvenile idiopathic arthritis (JIA) patients and healthy controls.

	Pre-vaccination		Post-vaccination			
	GMT	SP %	GMT	SP %	FI	SC %
Controls (n = 91)	12.4 (9.8–15.7)	20.9 (12.5–29.3)	250.8 (197–319.3)	95.6 (91.4–99.8)	20.3 (15.6–26.3)	95.6 (91.4–99.8)
JIA patients (n = 95)	10.6 (8.3–13.5)	20 (11.9–28.1)	215.8 (159.2–292.5)	88.4 (82–94.9)	20.4 (15–27.6)	**83.2* (75.6–90.7)**
JIA subtypes						
Oligoarticular (n = 24)	7.9 (5.9–10.7)	12.5 (0–26)	195.8 (110.2–348.1)	87.5 (74–101)	24.7 (13.6–44.7)	87.5 (74–101)
Polyarticular (n = 45)	11.7 (8–17.1)	22.2 (9.9–34.5)	198.5 (125.5–314)	88.9 (79.6–98.2)	17 (10.8–26.8)	**80* (68.2–91.8)**
RF-positive (n = 12)	22.4 (8.4–60.2)	25 (0–50.6)	285.1 (114.4–710.6)	91.7 (75.3–108)	12.7 (5.1–31.4)	**75* (49.4–100.6)**
RF-negative (n = 33)	9.2 (6.5–13.1)	21.2 (7–35.4)	174 (102.3–296)	87.9 (76.6–99.2)	18.9 (11.1–32.1)	**81.8* (68.5–95.2)**
Systemic (n = 18)	9.3 (5.5–15.5)	16.7 (0–34.4)	201.6 (102.4–396.7)	88.9 (73.9–103.8)	21.8 (11–42.9)	83.3 (65.6–101)
ERA (n = 8)	20 (5.8–68.5)	38 (1.6–73.4)	**538.2* (194.3–1490.8)**	87.5 (63–112)	26.9 (9.4–77)	87.5 (63–112)
DMARD/IS use						
Yes (n = 55)	10.5 (7.7–14.4)	16.4 (6.5–26.2)	230.6 (154.1–345.1)	89.1 (80.8–97.4)	21.9 (14.7–32.6)	85.5 (76.1–94.9)
No (n = 40)	10.7 (7.3–15.7)	25 (11.4–38.6)	197 (123.5–314.3)	87.5 (77.1–97.9)	18.4 (11.5–29.5)	**80* (67.4–92.6)**
MTX use						
Yes (n = 47)	11.1 (7.8–15.9)	17 (6.2–27.9)	211.7 (134.5–333.4)	87.2 (77.6–96.9)	19.1 (12.3–29.5)	**83* (72.1–93.8)**
No (n = 48)	10.1 (7.3–14.1)	22.9 (10.9–34.9)	219.8 (145.8–331.4)	89.6 (80.9–98.3)	21.7 (14.2–33.1)	**83.3* (72.7–94)**
Anti-TNF use						
Yes (n = 16)	11.4 (6.3–20.7)	18.8 (0–38.5)	306.4 (158.1–593.9)	100	26.9 (13.7–52.8)	93.8 (81.5–106)
No (n = 79)	10.4 (8–13.7)	20.3 (11.3–29.2)	201 (143.1–282.3)	**86.1* (78.4–93.8)**	19.2 (13.7–27)	**81* (72.3–89.7)**

GMT, Geometric mean titre; SP, seroprotection; FI, factor increase in GMT after vaccination; SC, seroconversion; RF, rheumatoid factor; ERA, enthesitis-related arthritis; MTX, methotrexate; TNF, tumour necrosis factor; DMARD, disease-modifying anti-rheumatic drug; IS, immunosuppressive drug.
Data are expressed as % or value (95% confidence interval).
* $p < 0.05$ compared to control group.

Table 2. Demographic data, juvenile idiopathic arthritis (JIA) subtypes, disease parameters, and treatment according to seroconversion in JIA patients.

	Seroconverted (n = 79)	Non-seroconverted (n = 16)	p
Demographic data			
Female gender	44 (55.7)	11 (68.7)	0.412
Current age (years)	14.7 ± 3.2	16.2 ± 2.7	0.086
Disease duration (years)	7.4 ± 4.5	8.4 ± 5.1	0.449
JIA subtypes			
Oligoarticular	21 (26.6)	3 (18.8)	0.754
Polyarticular	36 (45.6)	9 (56.3)	0.584
Systemic	14 (17.7)	3 (18.8)	1.000
ERA	8 (10.1)	1 (6.3)	1.000
Disease parameters			
Number of active joints	0 (0–16)	0 (0–28)	0.441
ESR (mm/1st h)	18 (1–83)	23 (2–55)	0.842
CRP (mg/dL)	1.8 (0.1–137.3)	1.9 (0.2–25.4)	0.505
Patient VAS, 0–100 mm	10 (0–80)	6 (0–80)	0.669
Physician VAS, 0–100 mm	10 (0–84)	12.5 (0–90)	0.718
CHAQ	0 (0–3)	0.125 (0–2)	0.588
Treatment			
Immunosuppressive drugs	47 (59.5)	8 (50)	0.582
Prednisone dose (mg/day)	5 (2.5–20)	–	–
MTX dose (mg/week)	25 (7.5–50)	30 (5–50)	0.661

ERA, Enthesitis-related arthritis; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; VAS, visual analogue scale; CHAQ, Childhood Health Assessment Questionnaire; MTX, methotrexate.
Data are expressed as number (%), mean ± standard deviation, or median (range).

Table 3. Disease activity parameters, patient and physician VAS and CHAQ of juvenile idiopathic arthritis (JIA) patients before and after vaccination.

Variable	Before vaccination	After vaccination	p
Disease activity			
Number of active joints	0 (0–28)	0 (0–18)	0.552
ESR (mm/1st h)	19 (1–83)	15 (0–83)	0.016
CRP (mg/dL)	1.9 (0.1–137.3)	2.7 (0.2–122.8)	0.073
Patient VAS, 0–100 mm	10 (0–80)	8.5 (0–80)	0.001
Physician VAS, 0–100 mm	10 (0–90)	6 (0–80)	0.002
CHAQ	0.123 (0–3)	0 (0–3)	0.058

VAS, Visual analogical scale; CHAQ, Childhood Health Assessment Questionnaire; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein.
Data are expressed as median (range).

Vaccine safety

Adverse events were reported by 42.1% patients and 44% controls ($p = 0.882$). No severe adverse event was reported during up to 3 weeks of follow-up. Only acute and mild arthralgia was significantly higher in JIA patients after vaccination compared to controls (12.6% vs. 2.2%, $p = 0.01$), with a median duration of 1 day (1–5) and median time for arthralgia appearance of 1 day (1–12) after vaccination. The most frequent adverse events in patients and controls were local pain (21.1% vs. 23.1%, $p = 0.86$), headache (14.7% vs. 19.8%, $p = 0.438$), and myalgia (15.8% vs. 6.6%, $p = 0.063$).

Discussion

To our knowledge this is the largest study in JIA patients demonstrating that the adjuvant-free influenza A H1N1/2009 vaccine induces a reduced but adequate humoral response probably independent of disease parameters and treatment. However, no influenza infections were recorded, so only surrogate endpoints (immunogenicity) were measured.

The strength of our study lies in the inclusion of an age-balanced healthy control group because vaccine immune response has a distinct pattern in children and adolescents (20); those aged < 9 years were excluded because they require two doses for adequate immunogenicity (20, 21). The inclusion of all JIA subtypes is, however, a limitation because clinical and genetic features as well as treatment and outcomes are not uniform in each subgroup of patients (22).

Importantly, for pandemic influenza vaccines to be approved in a paediatric population, all three current standards must be met (9–11). Therefore, despite a reduced immune response in JIA patients, this population fulfilled all of the three criteria indicating an effective

immune response. Similarly, a satisfactory immunogenicity was observed with seasonal influenza vaccination in previous studies with juvenile rheumatic diseases (12), including JIA patients (14). By contrast, our recent report evidenced reduced humoral immune response for the same vaccine in adult rheumatoid arthritis, particularly in those under MTX therapy (23).

Although influenza-like symptoms after immunization were evaluated, the incidence of post-vaccination influenza infection determined by collection of respiratory samples was not assessed. Therefore, the real reduction of influenza infection risk could not be calculated. The short-term efficacy demonstrated here suggests the necessity of a second boost of vaccination in non-responders. In fact, in a previous study with HIV-infected patients, a second dose of the pandemic H1N1/2009 influenza vaccine resulted in an additional increase in seroconversion rate (24).

In the present study, a reduced seroconversion rate was demonstrated in the polyarticular JIA group, which included patients most often treated with immunosuppressive therapies. However, the low number of non-seroconverted JIA patients as well as the limited number of patients on prednisone precludes a definitive conclusion about the possible deleterious effect of these drugs on immunogenicity.

Our JIA patients had lower seroconversion rates compared to controls, although only for the polyarticular onset was the differences statistically significant. The sample size for the other subtypes may be too small for the difference to reach statistical significance. Furthermore, a high post-vaccination GMT was observed in ERA JIA patients. The very limited number of ERA patients may hamper the interpretation of this finding.

We found that immunosuppressive therapy does not seem to influence the pandemic influenza vaccine antibody response in JIA patients, as also evidenced in adults rheumatoid arthritis and ankylosing spondylitis (25). Previous studies have also reported no effect of these drugs on the immunogenicity of seasonal vaccine in juvenile rheumatic diseases patients, including small numbers of JIA patients (12–14). On the contrary, in adult systemic lupus erythematosus patients, immunosuppressive drugs were associated with significantly diminished seroprotection and seroconversion rates for the pandemic vaccine (26). An overall deleterious effect of glucocorticoid therapy on this immune response was also observed in a large cohort of patients with juvenile rheumatic disease (15). The specific analysis of JIA population of the present study did not confirm this association probably because of the limited number of patients under this therapy.

A recent study has reported that disease parameters may impair the pandemic influenza vaccine response in adult lupus patients (27). The exclusion of hospitalized patients in the present cohort hampered the interpretation of the potential relevance of disease activity in the pandemic vaccine antibody response because of the low representation of these patients.

Disease safety was supported by our findings of a stable number of patients with arthritis and acute phase reactants throughout the study. Reinforcing this finding, previous studies with hepatitis, measles, mumps, and rubella vaccination did not show any increase in JIA activity parameters (28, 29). However, the lack of a non-vaccinated JIA control group in the present study hampers the accurate assessment of the effect of H1N1 vaccination on JIA disease activity itself.

The use of a non-adjuvant vaccine was chosen in this study to avoid any autoimmune-related diseases (30). Influenza A H1N1/2009 vaccine was well tolerated and safe in the JIA patients and no serious short-term adverse events were found, as was reported previously in a limited number of JIA patients who received seasonal influenza vaccine (12, 13). Only mild and acute arthralgia was observed in our JIA patients, as reported previously in our large study with 237 paediatric patients with autoimmune rheumatic diseases (15).

In conclusion, this prospective study of pandemic influenza A H1N1/2009 vaccination in JIA patients suggests adequate immunogenicity probably independent of therapy with no short-term harmful effect on the disease itself.

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